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Detection of Carcinogenicity Based on Mutagenicity in ARABIDOPSIS

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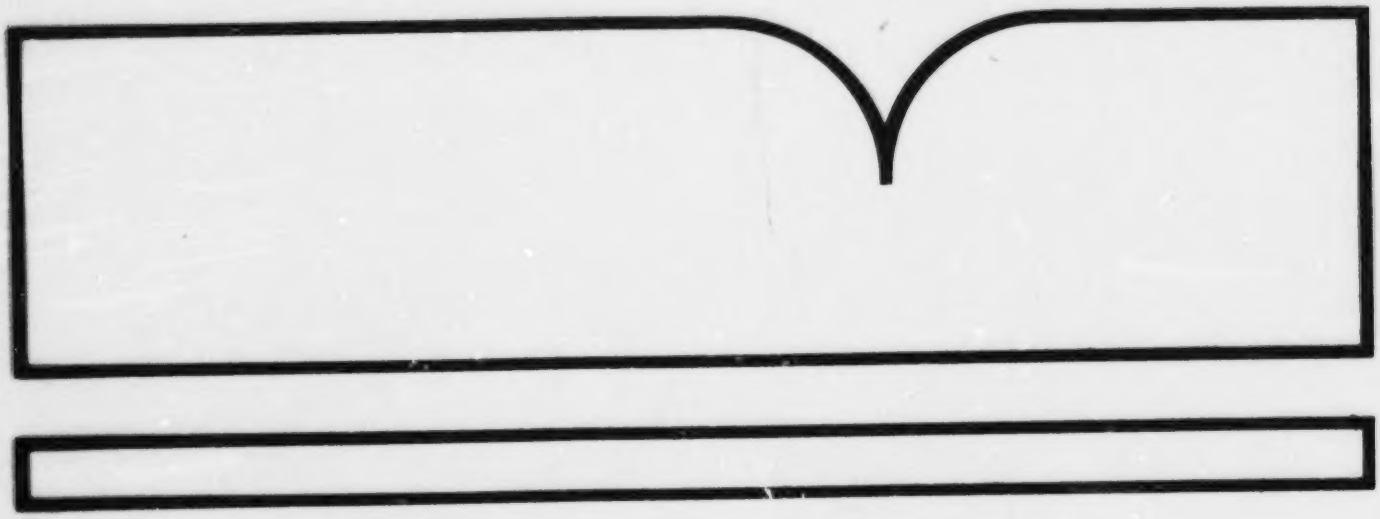

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DETECTION OF CARCINOGENICITY BASED ON MUTAGENICITY IN ARABIDOPSIS

by

George Redei and Gregoria N. Acedo
University of Missouri
Department of Agronomy
117 Curtis Hall
Columbia, MO 65211

EPA CR 807892

Project Officer

Shahbeg S. Sandhu
Genetic Toxicology Division
Health Effects Research Laboratory
Environmental Protection Agency
Research Triangle Park, NC 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

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16. ABSTRACT

Thirty-seven synthetic chemicals plus two mycotoxins were tested for mutagenicity in an Arabidopsis embryo system. The results of this test, prokaryotic repair tests, bacterial mutation assays, eukaryotic cell systems, and in vivo tests were compared to the carcinogenicity classifications of the chemicals.

Thirty-two of the thirty-seven chemicals tested were correctly identified as either mutagenic or nonmutagenic in the Arabidopsis assay. To compare these results with those of the other assays, we defined three criteria. "Sensitivity" indicated the percentage of tested carcinogens that were mutagenic in a system. Of 20 carcinogens tested in the Arabidopsis assay, 19 were mutagenic (sensitivity 95%). For the other assays, sensitivities ranged from 16% to 88%. "Specificity" indicated the percentage of noncarcinogens that were nonmutagenic in a system. Three of twelve noncarcinogens were nonmutagenic to Arabidopsis (specificity 25%). For the other assays, specificities ranged from 20% to 100%. The noncarcinogens pyrene, 4-acetylaminofluorene, 1-naphthylamine, isopropyl-N-(chlorophenyl) carbamate, azoxybenzene, and ascorbic acid were mutagenic in the Arabidopsis assay and other assays. "Accuracy" indicated the percentage of correctly identified chemicals based on the predicted classification. For the Arabidopsis assay, 22 of 32 chemicals were correctly identified (accuracy 69%). Overall accuracies for the other assays ranged from 57% to 62%.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

The overall objective of this report was to test thirty-seven chemicals and two mycotoxins for mutagenicity in an Arabidopsis embryo system. The results were compared with other tests described in this report.

F.Gordon Hueter,Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Within the framework of an international program, American, British, German and Japanese workers in 64 laboratories evaluated the mutagenic or related effects of 42 (or a somewhat reduced number) chemicals. The compounds were selected by expert panels as proven carcinogens, presumably harmless analogs of carcinogens or as being other types of noncarcinogens. The results of the studies were published in the volume EVALUATION OF SHORT-TERM TESTS FOR CARCINOGENS (F.J. de Serres & J. Ashby, eds., 1981, Elsevier/ North Holland, New York).

The biological assays included prokaryotic repair tests, Salmonella and Escherichia coli reversion and forward mutation systems, a wide variety of in vitro assays with eukaryotic cells and in vivo assays with Drosophila and mice. Although a wealth of valuable information was obtained, the results demonstrated that no single assay or any combination of assays are entirely satisfactory for the identification of carcinogens.

Therefore, we have tested with an Arabidopsis assay, 37 of the compounds involved in the program, plus two mycotoxins. This plant test is an in vivo system, capable of detecting mutations at a much larger number of loci than any other short-term methods involved in the original program. Based on the results obtained with 32 chemicals (the two mycotoxins ignored), we concluded that the sensitivity of the Arabidopsis assay was 0.9 (i.e. 90% of the carcinogens were positively identified). The sensitivity of the prokaryotic repair tests was within the range of 0.16 to 0.73, of the bacterial mutations assays between 0.27 to 0.76, of the eukaryotic cells systems varied between 0.08 to 0.88, and the sensitivity of the in vivo tests (Arabidopsis excluded) showed the ranges of 0.17 and 0.52.

The specificity of the Arabidopsis assay was 0.33 (i.e. 33% of the presumably noncarcinogens appeared negative). On the basis of our data and the results of several other assays, the classification of several compounds regarding carcinogenicity must be revised, however by comparison,the specificity of the other groups of assays showed the following ranges: prokaryotic repair tests 0.35 to 0.75, bacterial mutation assays from 0.59 to 0.82, eukaryotic systems 0.23 to 0.85, and that of the in vivo assays from 0.20 to 1.00.

The accuracy of the Arabidopsis assays was 0.69 (i.e. 69% of the compounds were classified as expected on the basis of the original carcinogenic versus noncarcinogenic properties assumed by the experts). The same parameter in the other systems was: prokaryotic repair 0.45 to 0.69, bacterial mutation 0.50 to 0.70, eukaryotic systems 0.31 to 0.81, and in the in vivo assays from 0.38 to 0.63.

On the basis of the overall results of these assays pyrene, 4-acetylaminofluorene, 2-naphthylamine isopropyl N(chlorophenyl)carbamate,and azoxybenzene can no longer be regarded as innocuous, and ascorbic acid also showed positive mutagenic properties in six Arabidopsis assays as well as in ten other assays.

In conclusion, on the basis of the chemicals involved in this international studies, the sensitivity of the Arabidopsis assay exceeded that of all other 64 assays, and its overall accuracy was among the 4 best. Accordingly, the Arabidopsis assay appears suitable for determining the carcinogenic properites of selected chemicals.

INTRODUCTION

Direct determination of carcinogenicity by mammalian or other animal assays is generally too expensive and too long in duration. Therefore, there is a great need for the rapid screening and identification of possible carcinogenic properties of chemicals which are synthesized now at a rate of 700 per year. The total number of chemicals currently in use is about 70,000. At this time, the gap between the number of chemicals adequately tested for carcinogenicity and the number of new compounds produced is widening.

In 1976, the British Medical Research Council initiated a program for testing the reliability of short-term indirect assays for the prediction and screening of carcinogenic chemicals. The United States National Institute of Environmental Health Sciences, the United States Environmental Protection Agency, the National Cancer Center Research Institute of Japan joined these efforts and eventually 64 laboratories participated in the program.

The chemicals were selected by experts on the basis of proven carcinogenicity in animal assays reported in the literature, and on the basis of personal experience and theoretical considerations of chemists. Some of the chemicals were paired, i.e. for a number of known carcinogens a structurally similar, but presumably noncarcinogenic analog, was selected on the basis of the best information then available. The array of the 42 compounds also included a number of other chemicals which were believed to be noncarcinogens.

The assays used in this study were grouped in four major classes: (i) prokaryotic repair, (ii) bacterial mutation, (iii) in vitro eukaryotic systems, and (iv) in vivo assays of higher organisms. The biological effects

of the compounds was determined by various means, e.g. genetic repair (survival), forward and reverse mutation in prokaryotes, fungi or mammalian cells, oncogenic transformation of cultured mammalian cells, gene conversion or mitotic recombination of fungi, sister-chromatid exchange, sex-linked recessive mutation in Drosophila, chromosomal aberrations of various types, etc.

None of the assays identified correctly all the carcinogens and all of the noncarcinogens. Therefore, the need for finding more accurate short-term assays remained. Our goal was to determine whether the Arabidopsis assay (Redei 1981, 1982) could be used effectively to supplement a battery of tests for the identification of carcinogens. The results of these Arabidopsis tests, and their evaluation in comparison with the outcome of the other assays are presented in the following pages.

MATERIAL AND METHODS

Arabidopsis thaliana (L.) Heynh., Columbia wild type was used for all the tests. Generally, we imbibed the seed in water at about 24°C for 24 hrs., then at the same temperature we treated the material for 15 hrs. with the chemicals dissolved in a nutrient mineral solution (E medium, commonly used for in vitro culture of this plant in our laboratory [Redei 1965]). Some of the compounds which did not readily dissolve in this mineral solution, were dissolved in various concentrations of dimethylsulfoxide (DMSO). Generally, we employed the chemicals in six different concentrations. The range of concentrations of the test compounds was determined by their toxicity to germination and/or survival or fertility of the plants. The treatment was followed by washing in running water for 4 hours before planting.

Unless otherwise stated, approximately 250 seeds (suspended in water) were planted with the aid of a blunted pasteur pipet on the surface of a "Promix" medium (Premier Brands, New Rochelle, NY 10804), moistened and placed in covered glass vessels (9.5 cm in diameter and 7 cm high). Before use, the medium was heated to 80°C in an oven for 1 hr to kill the contaminating microflora that frequently damaged the plants without this sterilization.

The cultures were kept in a growth chamber at 24°C and at a light intensity of 800 foot candle (GE high output, cool white fluorescent lights). Until germination, the vessels were covered by petri dishes. After germination the cover was removed, and the moisture content was maintained by periodic addition of distilled water.

Each experiment included a concurrent control in which the seed was exposed only to the solvent rather than to a chemical solution but in other respects the handling was identical to the treated material.

Previous studies demonstrated that in the mature seed of Arabidopsis the germline is represented by two diploid cells. Consequently, each plant is expected to reflect the genetic constitution of these two cells, and the descendant cell population in the inflorescence and fruits should show this dual origin when appropriate genetic markers are available. Thus if a mutation occurred in one of the cells of the germline, half of the fruits should show segregation of the embryos. The embryos developed on the plant emerged from the treated seed actually represent the M_2 generation because the meiosis and the following self-fertilization results in the segregation of the alleles and an independent assortment of the gametes, respectively.

Therefore, two fruits at the opposite side of the main stem, were

opened with the aid of sharp forceps after the normal cotyledons became green but before the seed coat became opaque. At this stage segregation for dominant and recessive genes affecting coloration of the embryos within the fruits was detected and recorded.

The data presented as "mutation %" represents the frequency of the fruits with pale or white embryos. From the actual figures the frequencies observed in the concurrent negative controls were subtracted. Thus, the "mutation %" expresses the frequency of the mutational events above the concurrent negative control. To expedite the screening, generally the number of mutant embryos within the fruits was not counted, only the number of the independent mutational events was determined. In each culture vessel 100 to 200 plants were analyzed.

From these data the overall mutation rate per genome can easily be deduced because each plant represents four genomes of the germline.

Some of the chemicals were supplied by EPA, others had been purchased from Sigma (St. Louis, MO 63178), azoxybenzene was obtained from Aldrich (Milwaukee, WI 53201). In several tables the chemicals are identified by name and code number. The code numbers designated the chemicals before their name was revealed to the participants in the program.

RESULTS

According to the original classification, among the 42 selected compounds there were 25 carcinogens proven by direct animal oncogenicity assays. The information concerning 9, 10-dimethylanthracene was not entirely convincing (Table 1). The presumable noncarcinogens could be divided into three categories. Class I appeared to be noncarcinogenic by the best available evidence whereas, class II and class III compounds had less good

Table 1.

Results of the *Arabidopsis* assays in comparison with the classification (Purchase et al. 1981) regarding the quality of evidence of carcinogenicity or noncarcinogenicity of the 42 compounds. I, II and III denote noncarcinogenicity class as defined in the text. The signs +, -, ? and o indicate mutagenic, nonmutagenic, questionable results and lack of information, respectively in the *Arabidopsis* assay.

Carcinogens	Noncarcinogens	Unclassified
4-Nitroquinoline-N-oxide o	Isopropyl-N-(3-chlorophenyl) carbamate I +	Pyrene +
Benzidine +		Anthracene -
4-Dimethylaminoazobenzene +		4-Acetylaminofluorene +
Benzo(a)pyrene +	4-Dimethylaminobenzene-4-sulphonic acid Na salt II o	Methionine -
β-Propiolactone +		Ascorbic acid +
9,10-Dimethylanthracene* ?	1,1,1-Trichloroethane II +	
Chloroform +	Dimethylformamide II +	
2-Acetylaminofluorene +	1-Naphthylamine II +	
Dimethylcarbamoyl chloride o	Diphenylnitrosamine II +	
2-Naphthylamine ?		
N-Nitrosomorpholine +	3-Methyl-4-nitroquinoline-N oxide III o	
Urethane +		
Methylazoxymethanol acetate +	3,3',5,5'-Tetramethylbenzidine III ?	
DL-Ethionine +	γ-Butyrolactone III ?	
Hydrazine sulphate +	Dinitrosopentamethylene tetramine III o	
Hexamethylphosphoramide ?		
Ethylenethiourea +	Azoxybenzene III +	
Diethylstilbestrol +	Sucrose III -	
Safrole +		
Cyclophosphamide +		
Epichlorohydrine +		
3-Aminotriazole -		
4,4'-Methylenebis(2-chloroaniline) +		
o-Toluidine hydrochloride +		
Auramine (technical) +		
Total: 25	Total class I: 1 class II: 5 class III: 6	Total: 5

*Data not convincing as to carcinogenicity

Table 2.
Mutagenic effect of 40 chemicals in the Arabidopsis embryo assay.

Code No.	Chemical	Conc. %	Mutations (%) Exceeding Concurrent Negative Control		
			I	II	III
8	4-nitroquinoline-N-oxide	NT			
15	3-methyl-4-nitroquinoline-N-oxide	NT			
11	benzidine	0.05	1.4		
		0.20	1.6		
2	3,3',5,5'-tetramethylbenzidine	0.50	5.3		
1	4-dimethylaminoazobenzene	0.05	1.0		
		0.05	2.5		
		0.10	3.0		
		1.00	1.0		
10	dimethylaminoazobenzene-4-sulphonic acid Na salt	NT			
13	benzo(a)pyrene	0.01	6.6		
		0.02	2.3		
		0.04	7.1		
		0.08	10.3		
		0.10	10.1		
		0.20	7.6		
5	pyrene	0.05	0		
		0.10	3.6		
		0.20	4.6		
		0.50	1.6		
		1.00	10.2		
		2.00	7.0		
29	β-propiolactone	0.05	3.0		
		0.10	4.0		
		0.20	10.5		
		0.50	no germination		
21	γ-butyrolactone	0.05	0.7		
		0.10	0		
		0.20	1.0		
		0.50	0.7		
		1.00	0.7		
		2.00	1.1		
39	9,10-dimethylanthracene	0.01	0.5		
		0.02	0		
		0.05	0		
		0.10	0.5		
		0.20	2.0		
18	anthracene	0.01	0		
		0.02	0		
		0.05	0		
		0.10	3.0		
		0.20	0.5		
		0.40	0		
6	chloroform	0.05	0	-	-
		0.10	4.6	7.5	0
		0.20	0	3.0	0.6
		0.50	0	-	-
		1.00	0	3.9	-
		3.00	0	-	-

Table 2 (continued, 2)

Code No.	Chemical	Conc. %	Mutations (%) Exceeding Concurrent Negative Control		
			I	II	III
30	1,1,1-trichloroethane	0.05	0	-	
		0.10	0	32.0	
		0.20	3.4	4.5	
		0.50	0	-	
		1.00	0	4.0	
		3.00	no survivors		
12	2-acetylaminofluorene	0.05	0		
		0.10	0		
		0.20	3.4		
		0.50	0		
		1.00	4.8		
		2.00	1.4		
3	4-acetylaminofluorene	0.05	0.3		
		0.10	0		
		0.20	0		
		0.50	0		
		1.00	2.8		
		2.00	5.6		
32	dimethylcarbamoyl chloride	NT			
24	dimethylformamide	0.05	5.5	-	
		0.10	6.0	4.5	
		0.20	3.5	-	
		0.50	0		
		1.00	0		
		2.00	0.5		
9	2-naphthylamine	0.005	0		
		0.01	0		
		0.02	0.5		
		0.04	0		
		0.08	0		
		0.10	0.8		
		0.20	0.5		
4	1-naphthylamine	0.025	0.5		
		0.05	1.0		
		0.10	1.0		
		0.25	2.7		
		0.50	1.4		
27	nitrosomorpholine	0.013	2.0		
		0.025	1.5		
		0.05	1.5		
		0.10	2.0		
		0.25	3.0		
17	diphenylnitrosamine	0.025	0		
		0.05	4.0		
		0.10	3.5		
		0.20	7.0		
		0.50	0.5		
		1.00	4.0		
19	dinitrosopentamethylene tetramine	NT			

Table 2 (continued, 3)

Code No.	Chemical	Conc. %	Mutations (%) Exceeding Concurrent Negative Control I	II	III
31	urethane	0.05	3.4		
		0.10	0.6		
		0.20	6.0		
		0.50	0.5		
		1.00	2.7		
		2.00	3.1		
28	isopropyl N(3-chlorophenyl)carbamate	0.05	6.1		
		0.10	1.6		
		0.20	0		
		0.50	0		
		1.00	0.5		
		2.00	0		
37	methylazoxymethanolacetate	0.01	0		
		0.02	7.0		
		0.04	33.0		
		0.10	lethal		
		0.20	lethal		
		0.60	lethal		
33	azoxybenzene	0.05	0	0	
		0.10	6.2	11.2	
		0.20	0.5	1.3	
		0.50	5.5	-	
		1.00	0		
		3.00	lethal		
25	DL-ethionine	0.05	3.5		
		0.10	2.5		
		0.20	4.5		
		0.50	3.5		
		2.00	2.0		
22	methionine	0.10	1.1		
		0.20	0		
		0.50	0		
		2.00	0		
14	hydrazine sulphate	0.05	5.3		
		0.10	6.1		
		0.20	1.2		
		0.50	0		
		1.00	3.8		
		2.00	3.1		
16	hexamethylphosphoramide	0.05	0		
		0.10	0		
		0.20	0		
		0.50	NA		
		1.00	NA		
		2.00	NA		
20	ethylenethiourea	0.05	2.0		
		0.10	0		
		0.20	2.5		
		0.50	0.5		
		1.00	0.5		
		2.00	0.5		
7	diethylstilbestrol	0.05	no germin.	3.0	
		0.10	"	0	
		0.20		2.3	
		0.50	"	1.1	
		1.00	"	0	
		2.00	"	no germin.	

Table 2 (continued, 4)

Code No.	Chemical	Conc. %	Mutations (%) Exceeding Concurrent Negative Control I	II	III
23	safrole	0.10	0		
		0.20	2.3		
		0.40	10.1		
		1.00	7.3		
		2.50	0		
34	cyclophosphamide	0.05	0	-	
		0.10	3.0	3.5	
		0.20	4.0	14.5	
		0.50	1.0	4.0	
		1.00	2.0	-	
		2.00	5.5	-	
26	epichlorohydrine	0.001	6.5		
		0.0001	3.2		
35	3-aminotriazole	0.0001	0		
		0.0010	2.0		
		0.0100	0.1		
		0.0500	lethal		
36	4,4'-methylenebis(2-chloroaniline)		3.2		
			1.8		
			2.70		
			0		
			0		
			0		
41	toluidine	0.05	1.1		
		0.10	1.0		
		0.20	8.1		
		0.50	0.7		
		1.00	lethal		
42	auramine	0.05	15.8		
		0.10	5.6		
		0.20	5.2		
		0.50	3.1		
		1.00	13.2		
		2.00	0		
38	sucrose	0.05	0		
		0.10	0.9		
		0.20	0		
		0.50	0		
		2.00	0		
40	ascorbic acid	0.05	7.8		
		0.10	4.4		
		0.20	6.2	see additional data	
		0.50	0	in Table	
		1.00	6.6		
		2.00	4.2		
Aflatoxin B1 T2 mycotoxin	see data on Table 11				

Table 3. List of chemicals involved in the international project, carcinogenicity information (+ = carcinogen, - = noncarcinogen), and the classification by the Arabidopsis assay (• = mutagen, o = nonmutagen, ? = inconclusive data, NT = not tested).

Chemical	Carcinogenicity	Arabidopsis Assay
4-Nitroquinoline-N-oxide	+	NT
3-Methyl-4-nitroquinoline-N-oxide	-	NT
Benzidine	+	•
3,3',5,5'-Tetramethylbenzidine	-	?
4-Dimethylaminoazobenzene	+	•
4-Dimethylaminoazobenzene-4-sulphonic acid Na salt	-	NT
Benzo(a)pyrene	+	•
Pyrene	-	•
β-Propiolactone	+	•
γ-Butyrolactone	-	?
9,10-Dimethylanthracene	+	?
Anthracene	-	o
1,1,1-Trichloroethane	-	•
Chloroform	+	•
2-Acetylaminofluorene	+	•
4-Acetylaminofluorene	-	•
Dimethylcarbamoyl chloride	+	NT
Dimethylformamide	-	•
2-Naphthylamine	+	?
1-Naphthylamine	-	•
N-Nitrosomorpholine	+	•
Diphenylnitrosamine	-	•
Dinitrosopentamethylene tetramine	-	NT
Urethane	+	•
Isopropyl N(3-chlorophenyl)carbamate	-	•
Methylazoxymethanolacetate	+	•
Azoxybenzene	-	•
DL-Ethionine	+	•
Methionine	-	o
Hydrazine sulphate	+	•
Hexamethylphophoramide	+	?
Ethylenethiourea	+	•
Diethylstilbestrol	+	•
Safrole	+	•
Cyclophosphamide	+	•
Epichlorohydrin	+	•
3-Aminotriazole	+	o
4,4'-Methylenebis(2-chloroaniline)	+	•
o-Toluidine	+	•
Auramine	+	•
Sucrose	-	o
Ascorbic acid	-	•

evidence for noncarcinogenicity in this order. Five compounds were left unclassified by the expert panels because the evidence then available regarding carcinogenicity was unclear, yet they were supposed to be noncarcinogens. Table 1 lists these compounds and shows their classification as well as the results of the Arabidopsis assays. The sign + indicates mutagenicity, - nonmutagenicity, ? stands for ambiguity of the information obtained in Arabidopsis and o indicates lack of data.

Most of the compounds were tested in six different concentrations and they were rated as mutagenic in Arabidopsis if at least in two concentrations the observed frequency of mutation exceeded that in the concurrent negative control. When only one concentration of treatment yielded mutations above the spontaneous level, the judgement concerning mutagenic properties was suspended until more information can be obtained. Five of the compounds belong now to this category and these are identified by a ? sign. Four compounds failed to increase the spontaneous frequency of the mutational events and these are marked by a - sign. The detailed data are shown in Table 2.

Table 3 is the summary of the results obtained with Arabidopsis. If we accept the original classification of these selected chemicals, i.e. 20 of of the compounds tested can be expected to show positive mutagenic effect in Arabidopsis. Actually 18/20 were classified mutagenic on the basis of the Arabidopsis data. In other words, the sensitivity of the assay (the true positive fraction) appears to be 0.90.

We have tested also 12 presumably noncarcinogenic compounds but only 4/12 appeared nonmutagenic in the Arabidopsis assay, and most of them induced mutations at a detectable rate. Accordingly, the specifity of our assay

Table 4. Identification of the test systems represented by numbers on the Figures 1 to 21.

REPAIR TESTS

Rec. : 21 B. subtilis
22, 23, 24 E. coli
Pol A: 25 E. coli
Inductest: 26, 27 E. coli λ

BACTERIAL MUTATION

Ames test: 1 - 11 Salmonella
Reversion: 12 & 18 Salmonella his, E. coli trp
13 Salmonella his/norharman
14 Salmonella his/heparin
15 Salmonella azaG resist.
16 & 17 Salmonella, fluctuation
19 E. coli trp^-
20 E. coli, galR, arg, nad, leu forward

EUKARYOTIC SYSTEMS

Degranulation: 28 & 29
Nuclear enlargement: 31 human fibroblasts
Reversion Saccharomyces (ade2-1, arg4, lys1 trp5, his1, hom3: 32
Schizosaccharomyces, ade, forward 33
Mitotic recomb., repair, Saccharomyces 34
35, 37, 38 Saccharomyces mitotic gene conversion
36 Saccharomyces aneuploidy
39 Saccharomyces repair
40, 41 UDS, human fibroblasts
42 UDS HeLa
43,44, 45 SCE in vitro, CHO
46 Chromosome structure, rat cells
47 Mouse lymphoma, TK forward
48 CHO aprt/+, tk/+
49, 50 CHO HGPRT
51 CHO aneuploid line V79 HGPRT
52 Diphteria tox. resist, Human fibroblast
53, 54 BHK transformation
55 MLV integration

IN VIVO SYSTEMS

56,57, 58 Sex-linked recessive lethal Drosophila
59 SCE mouse
60 micronucleus, mice bone marrow
61, 62 micronucleus, mice erythrocytes
63 sperm abnormalities, mice

ARABIDOPSIS mutant embryos

appeared to be 4/12, i.e. 0.33. Some of these supposedly noncarcinogens such as pyrene was mutagenic in all except at the lowest concentration tested, and ascorbic acid showed mutaganicity in several concentrations in seven independent trials.

Our results with Arabidopsis confirmed the original carcinogenicity classification with 22/32 compounds. In other words, the accuracy of our test by these criteria appeared to be 0.69 (22/32).

Since most of these compounds had been tested earlier in four major types of systems, and within each category a variety of assays and different organisms were employed, a comparative overview seems interesting. An identification of the methods of the 63 other assays is provided in brief in Table 4. Several of these assays used the same or almost the same procedures for testing, e.g. 11 laboratories relied on the Salmonella/ microsome method of Ames. Three experiments, each, employed rec mutants of E. coli, mitotic gene conversion in Saccharomyces, sister chromatid exchange (SCE) in Chinese hamster ovary cells (CHO), and sex-linked recessive lethal assays in Drosophila melanogaster, respectively. Some other types of tests were also used more than ones and by different investigators. In spite of the identity of the batches of chemicals and the great similarity between some of the tests, the results show considerable variation (Figures 1 to 21).

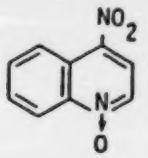
These charts were borrowed from the summary of Ashby (1981) and the information available from our experiments was suplemented in a heavy solid column at the right side of the charts; this column is identified also with an A. In these charts the experiments are identified in the four categories by the same numbers as in Table 4. The height of the column was chosen on the basis of the overall accuracy of the individual assays. In these reports,

accuracy is defined as the fraction of the correct results. Correct is the result if an originally supposed carcinogen showed a positive response (e.g. mutation) in a test or when a presumed noncarcinogen evoked no response (e.g. was nonmutagenic). The responses corresponding to the expectation are represented by solid columns, and the unexpected responses are shown by the cross-hatched columns (except in Arabidopsis where the bars are always solid black). At the right corners the chemical formula of the compounds is displayed along with the carcinogenicity information supplied by the panel before these assays began. The solid black circle denotes carcinogenicity, the roman numerals I, II, III indicate noncarcinogenicity with decreasing firmness of the information available. U indicates that the compound was presumed to be a noncarcinogen, however it could not be safely classified as such.

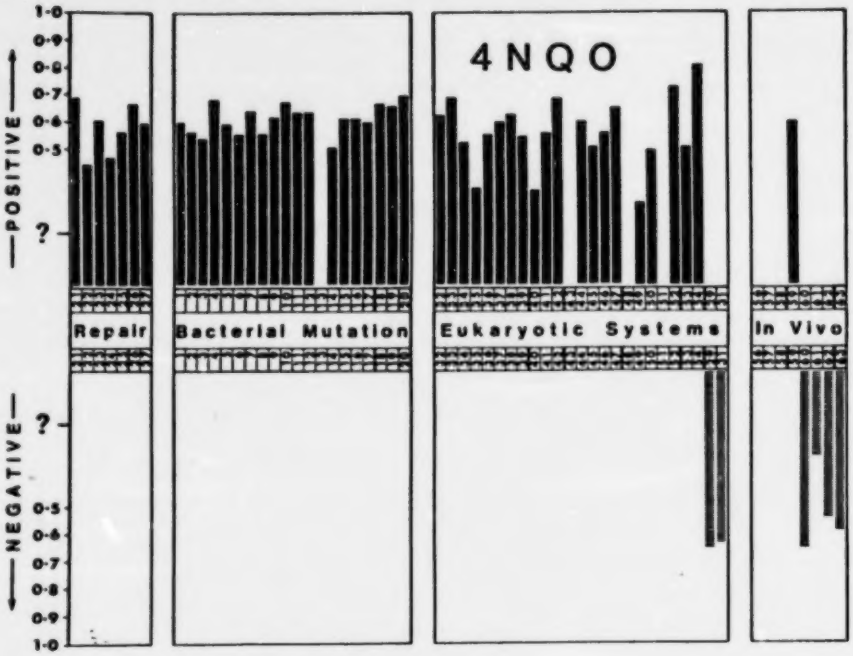
In these charts structurally similar chemical compounds are paired when possible. The carcinogen 4-nitroquinoline-N-oxide and the class III noncarcinogen analog were not tested yet with Arabidopsis. Most of the tests confirmed the expectation, namely nitroquinoline-N-oxide evoked the response in the test as carcinogens do, but the analog behaved as supposed for a noncarcinogen (Fig. 1).

The response of the test systems to the pair benzidine (carcinogen) and tetramethylbenzidine (class III noncarcinogen) was in a somewhat less good agreement with the expectation. Among the 63 tests with benzidine, 20 gave results contrary to expectation. The Arabidopsis assay correctly identified this carcinogen. The expectations were better met with tetramethylbenzidine in as much as only 7/53 tests indicated carcinogenicity for this noncarcinogen. The Arabidopsis assay gave inconclusive result and

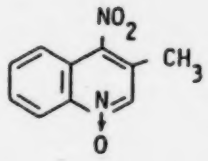
Figure 1.



4-Nitroquinoline-N-oxide

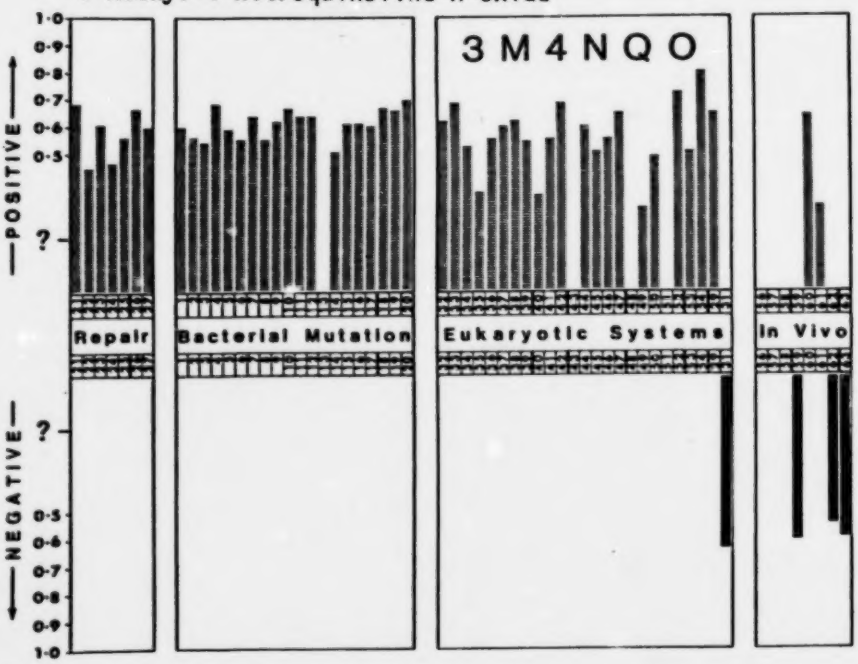


A



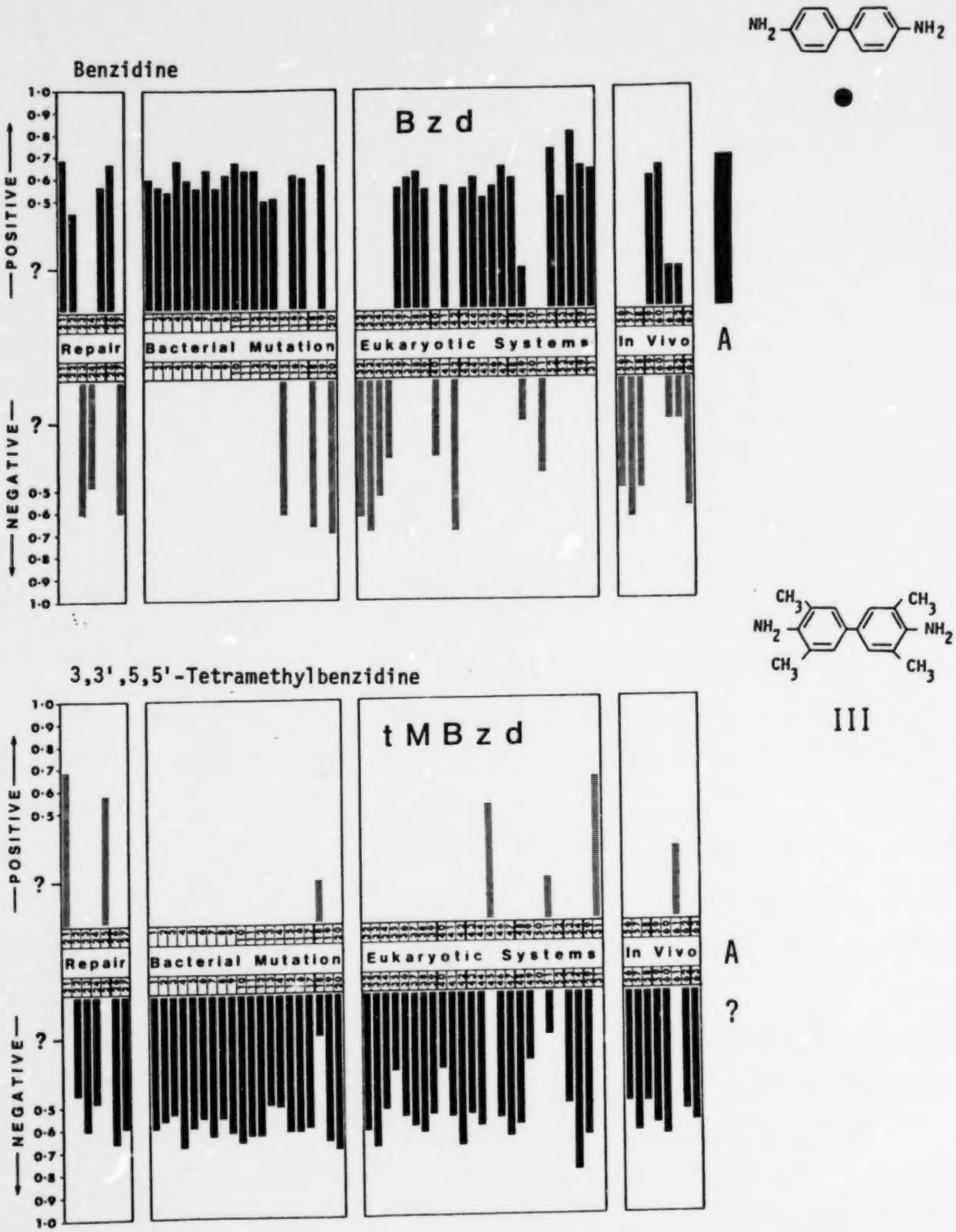
III

3-Methyl-4-nitroquinoline-N-oxide



A

Figure 2.


Benzidine
Bzd
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
3,3',5,5'-Tetramethylbenzidine
tMBzd
A
?

III

Figure 3.

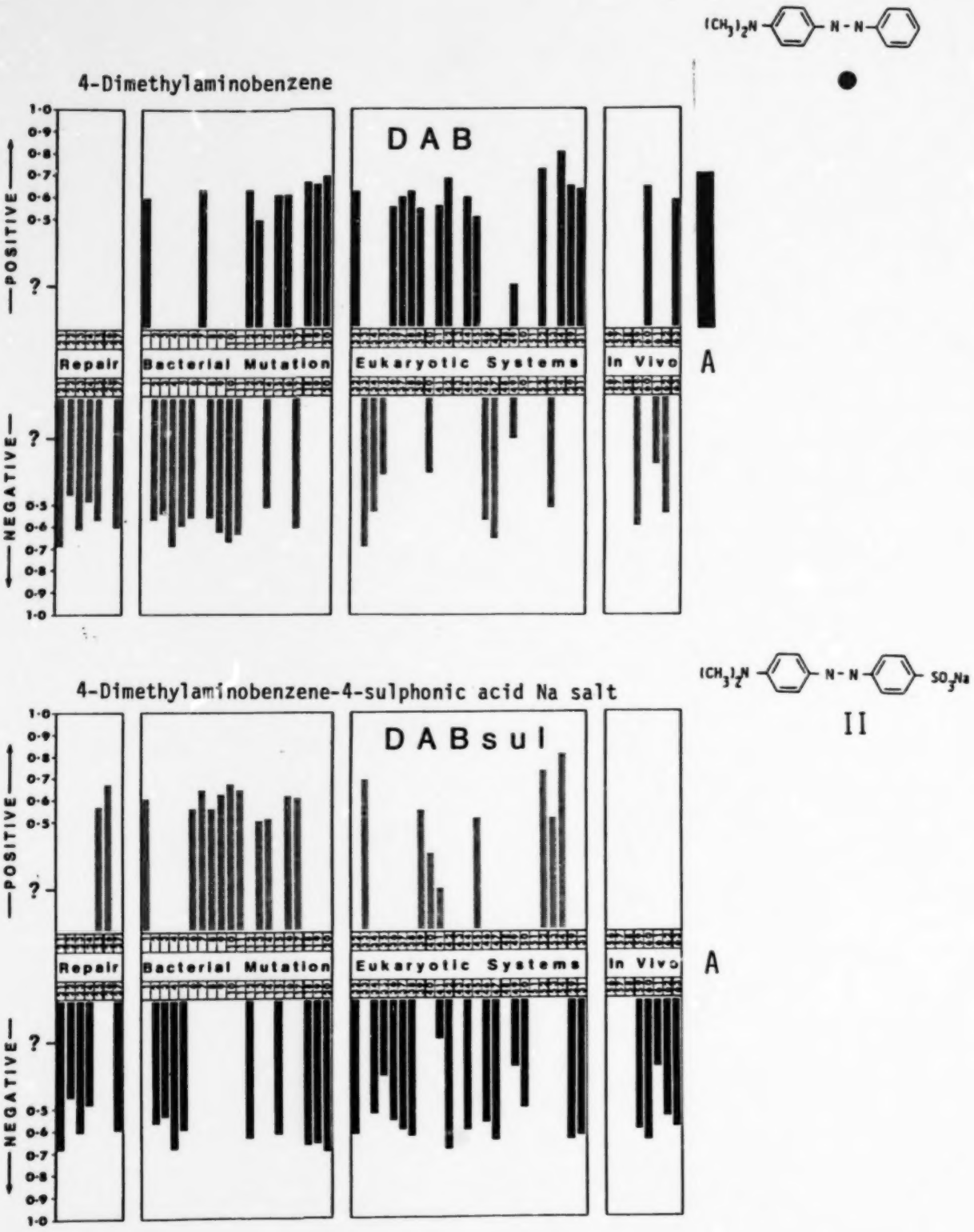

4-Dimethylaminobenzene
DAB
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
4-Dimethylaminobenzene-4-sulphonic acid Na salt
DABsul
II

Figure 4.

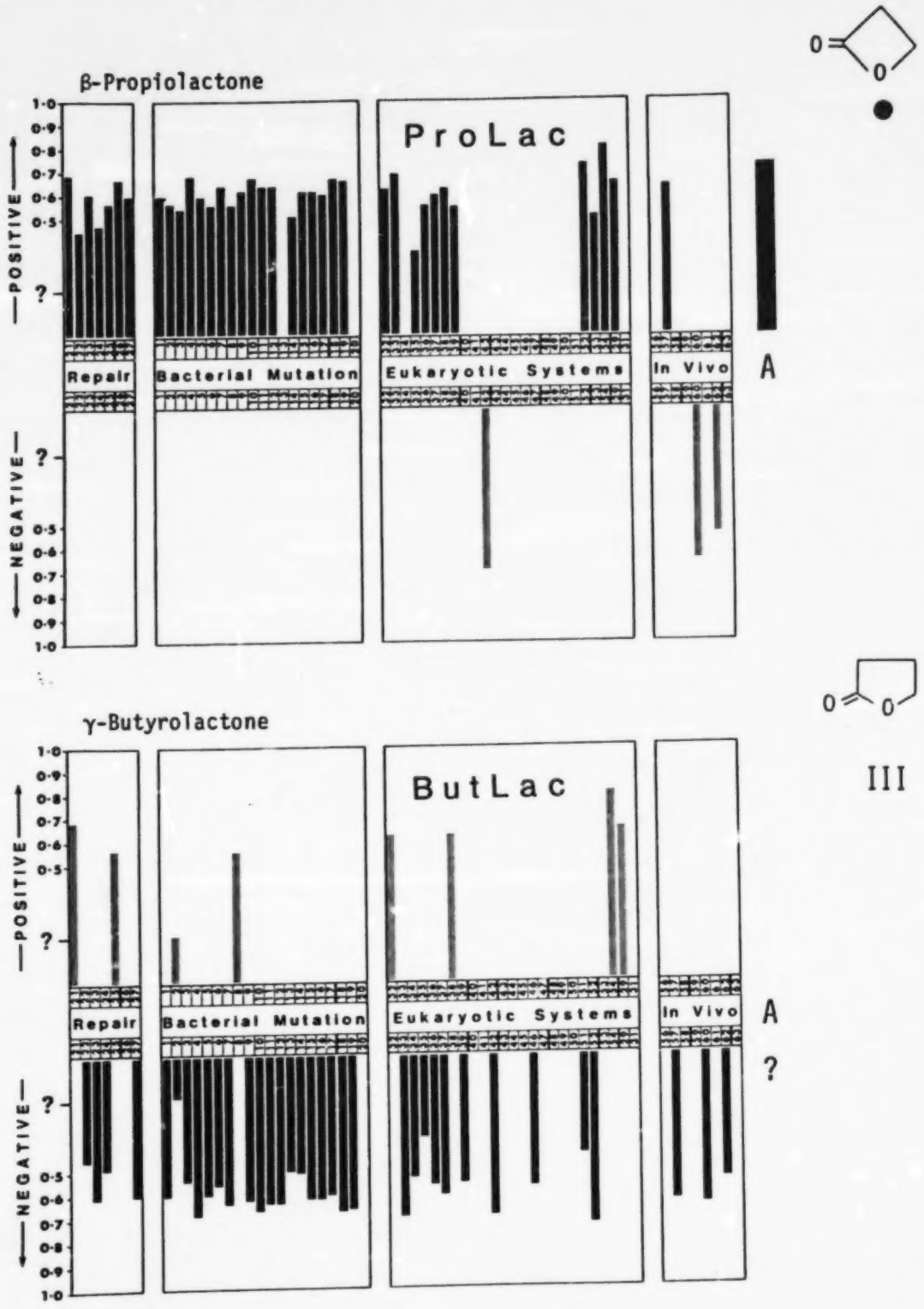

β-Propiolactone
ProLac
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
γ-Butyrolactone
ButLac
III
?

Figure 5.

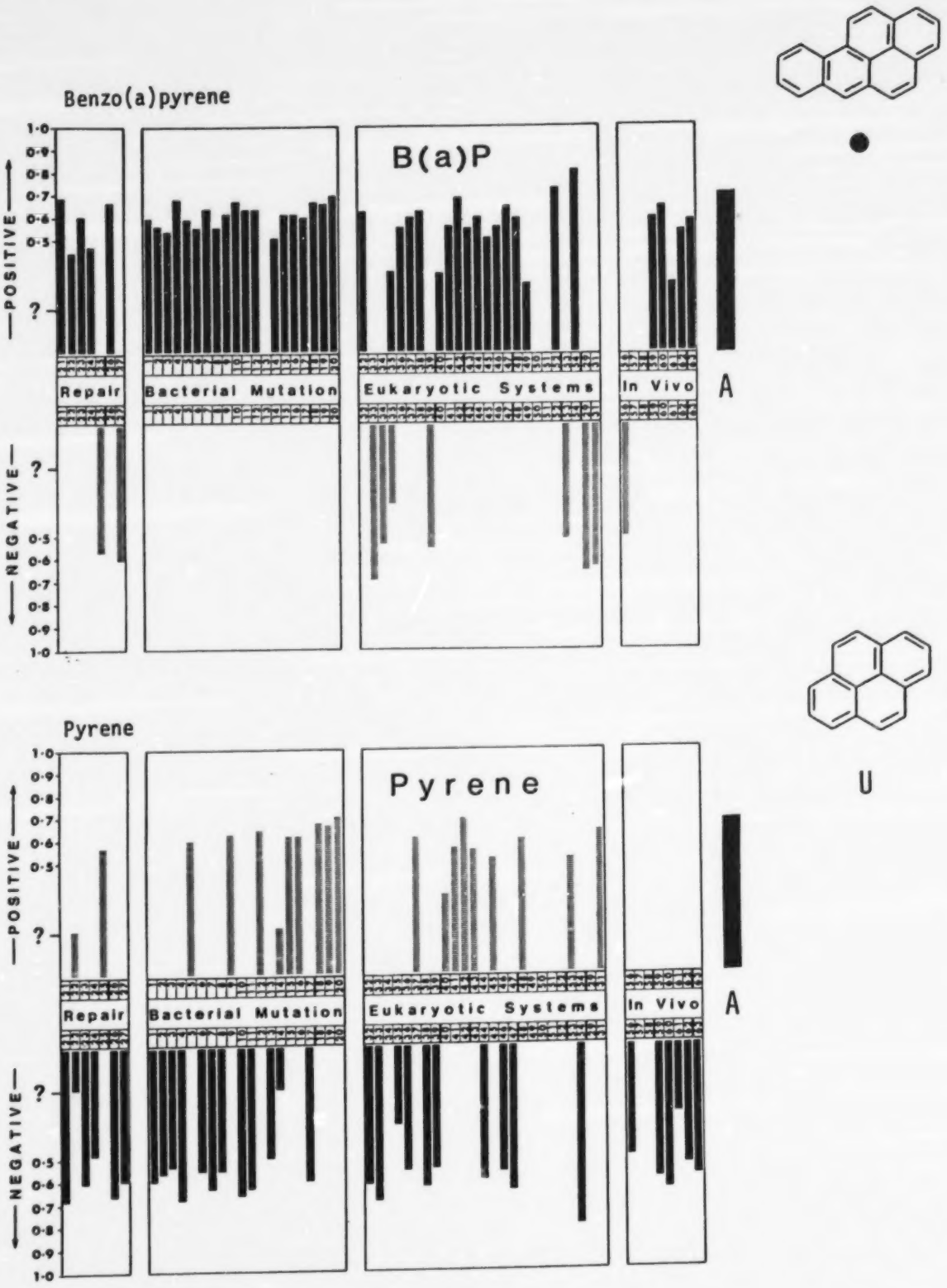

Benzo(a)pyrene
B(a)P
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Pyrene
Pyrene
U

Figure 6.

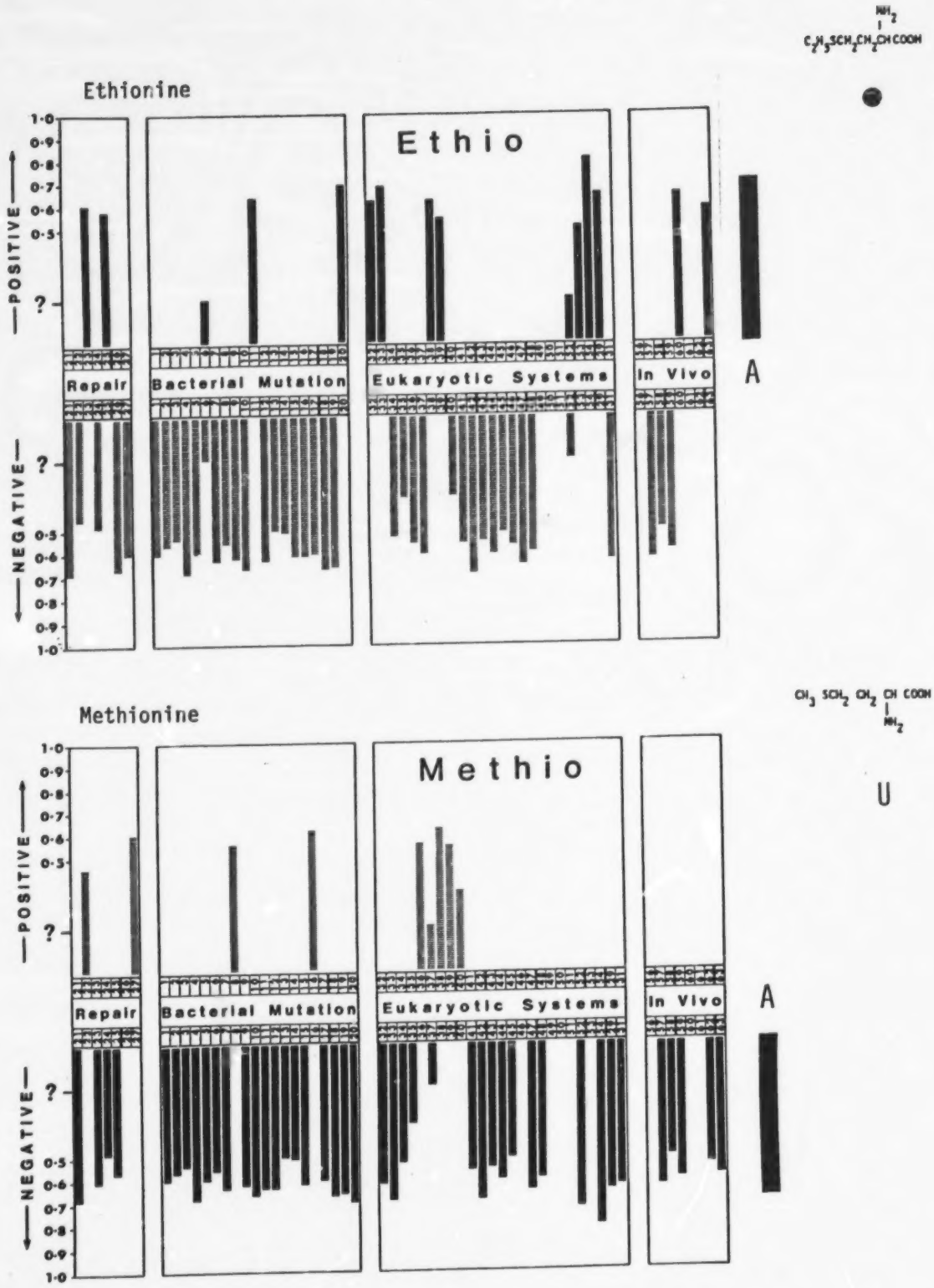

Ethionine
Methionine
Ethio
Methio
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
POSITIVE
NEGATIVE
?
1·0
0·9
0·8
0·7
0·6
0·5
A
U

Figure 7.

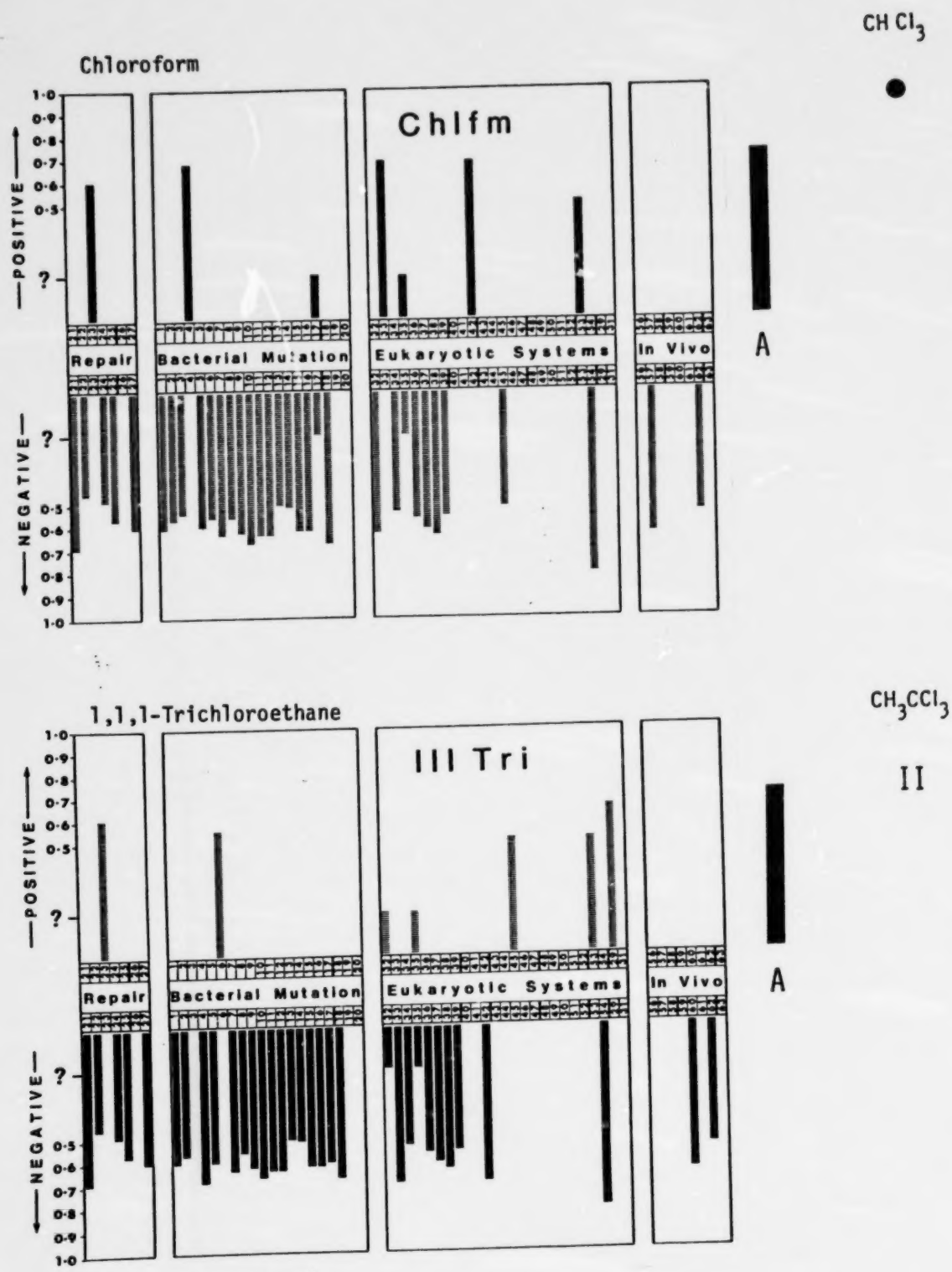

Chloroform
CH Cl3
Chlfm
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
1,1,1-Trichloroethane
CH3CCl3
III Tri
II
A

Figure 8.

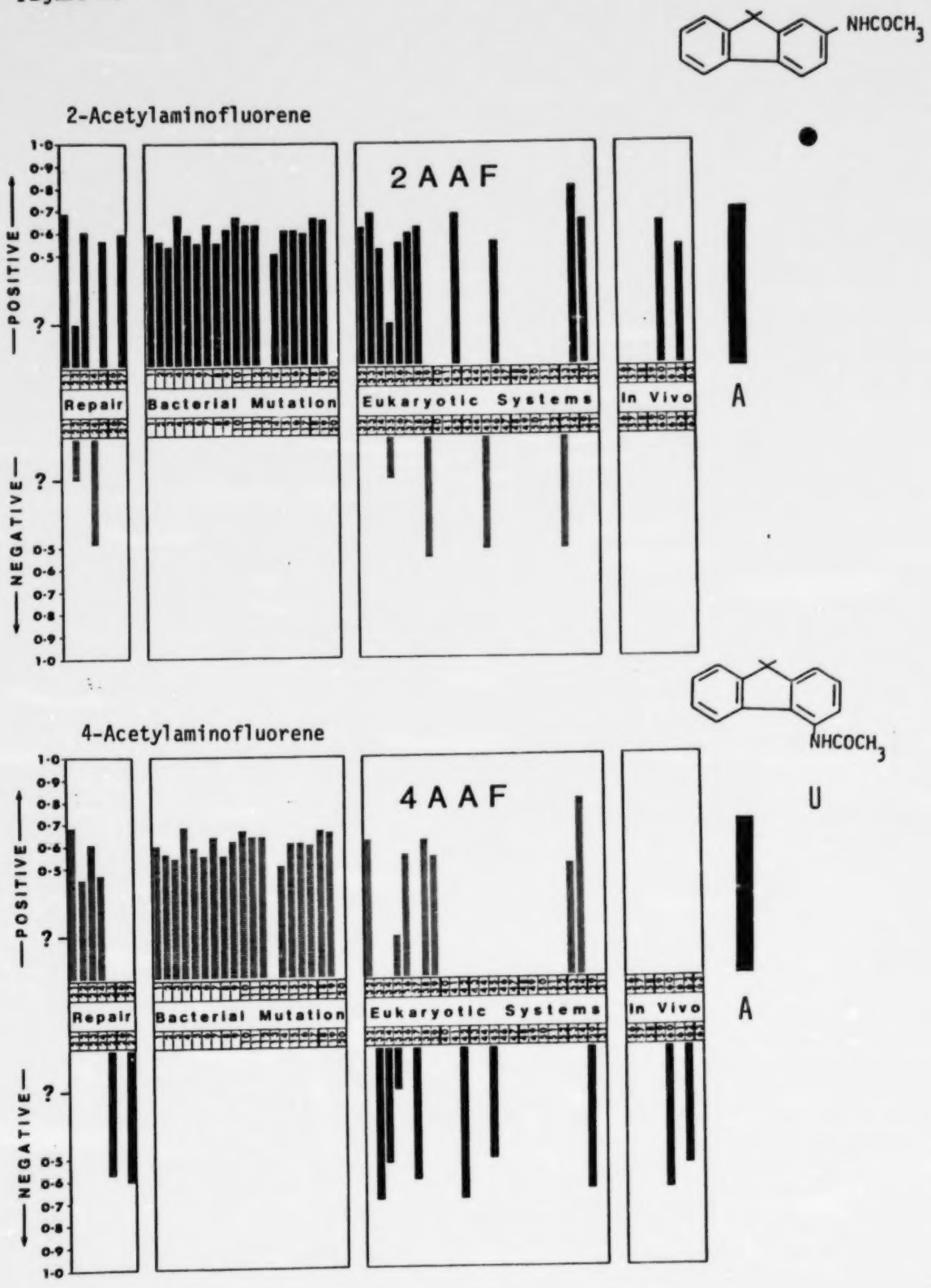

NHCOCH3
2-Acetylaminofluorene
2AAF
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
NHCOCH3
4-Acetylaminofluorene
4AAF
U
A

Figure 9.

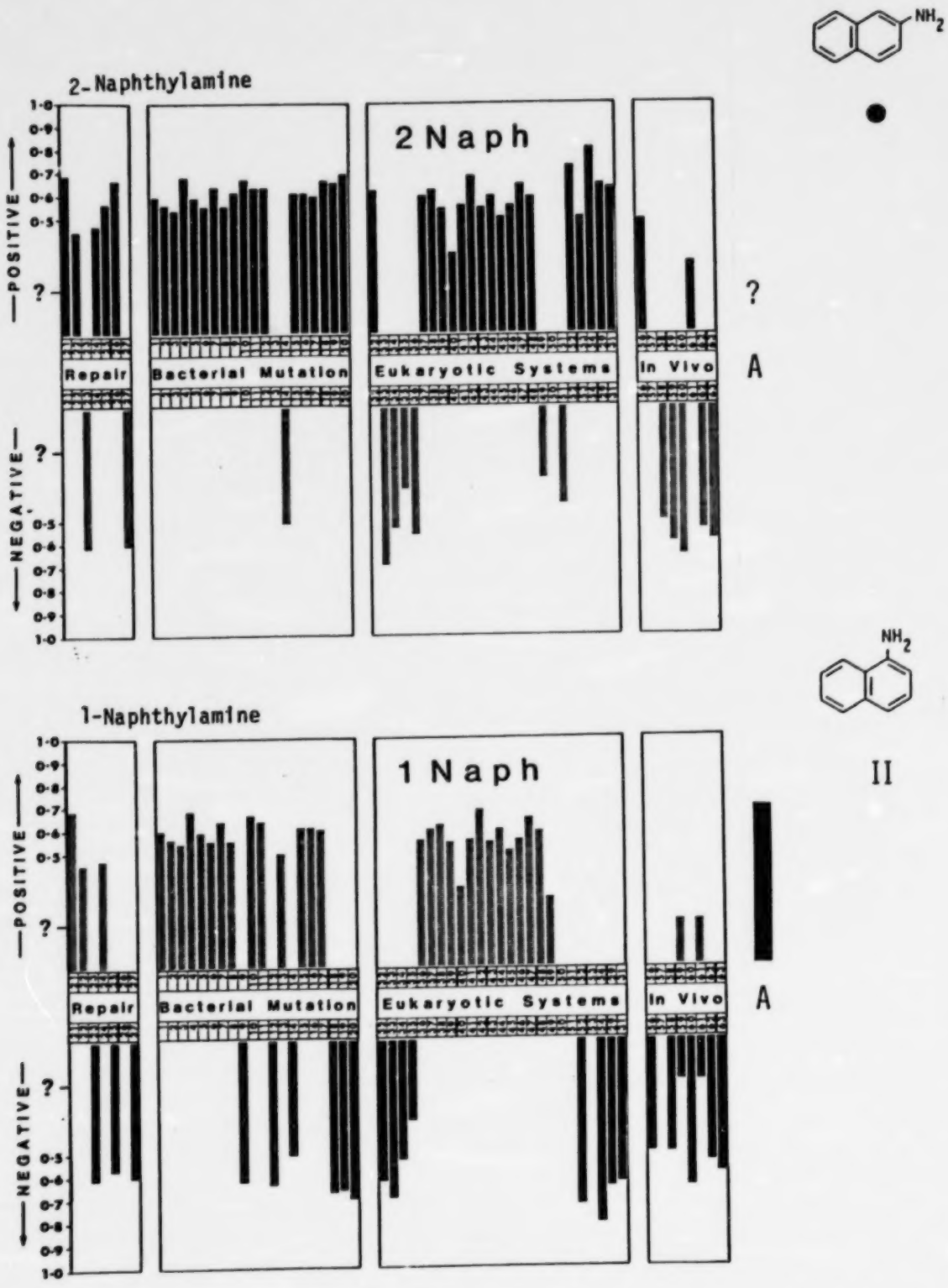

2-Naphthylamine
2 Naph
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
?
A
1-Naphthylamine
1 Naph
II

Figure 10.

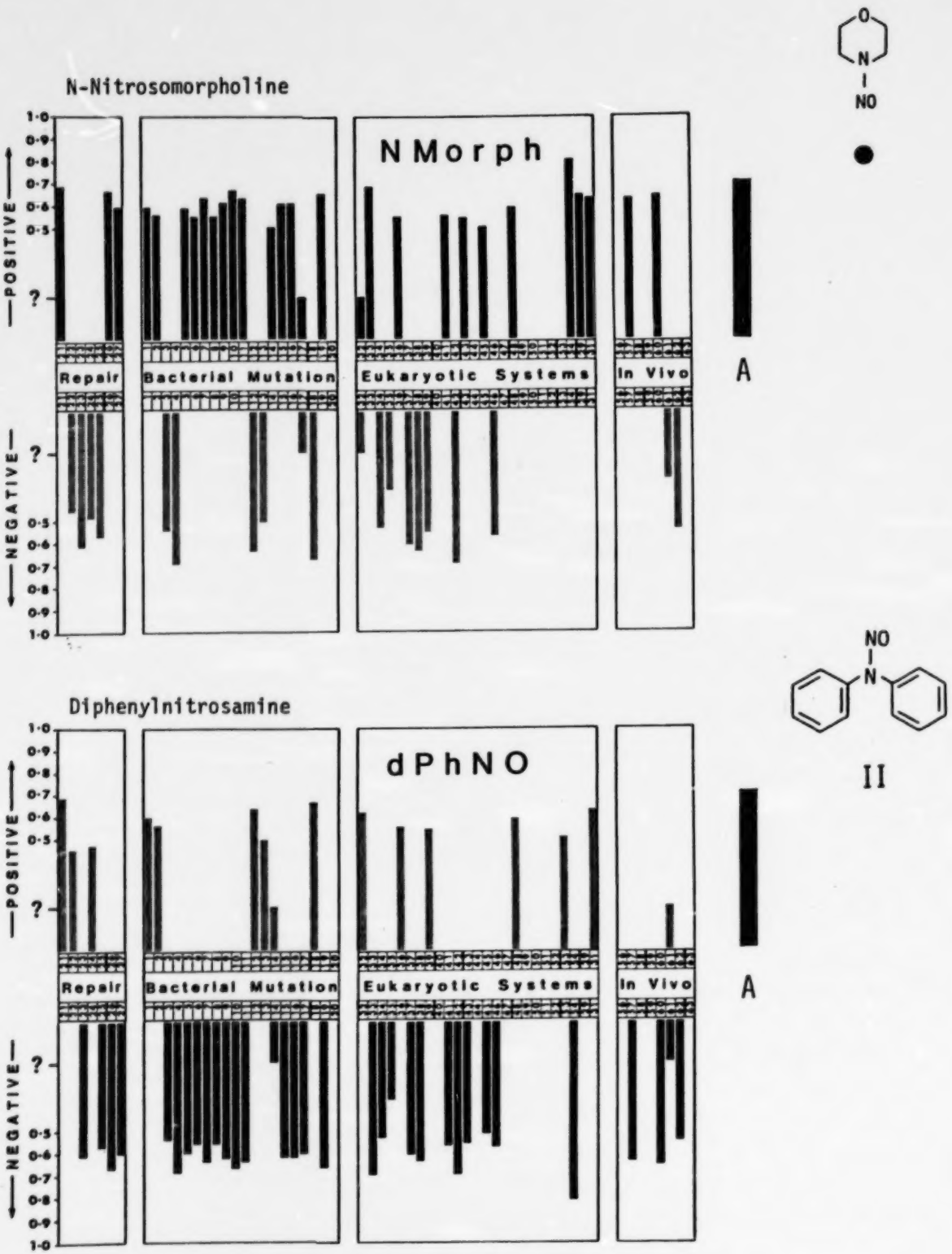

N-Nitrosomorpholine
O
N
NO
NMorph
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Diphenylnitrosamine
NO
N
II
dPhNO
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A

Figure 11.

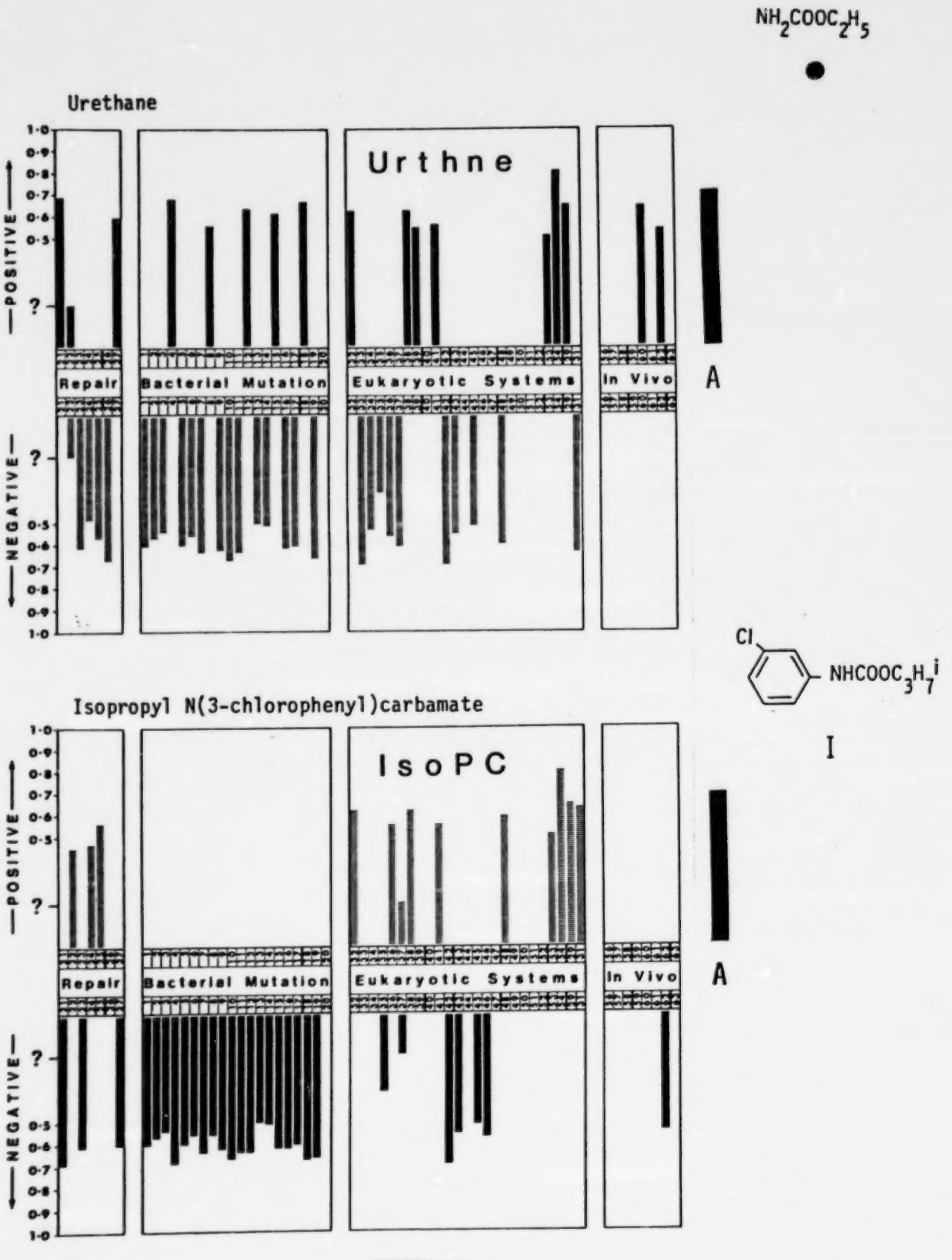
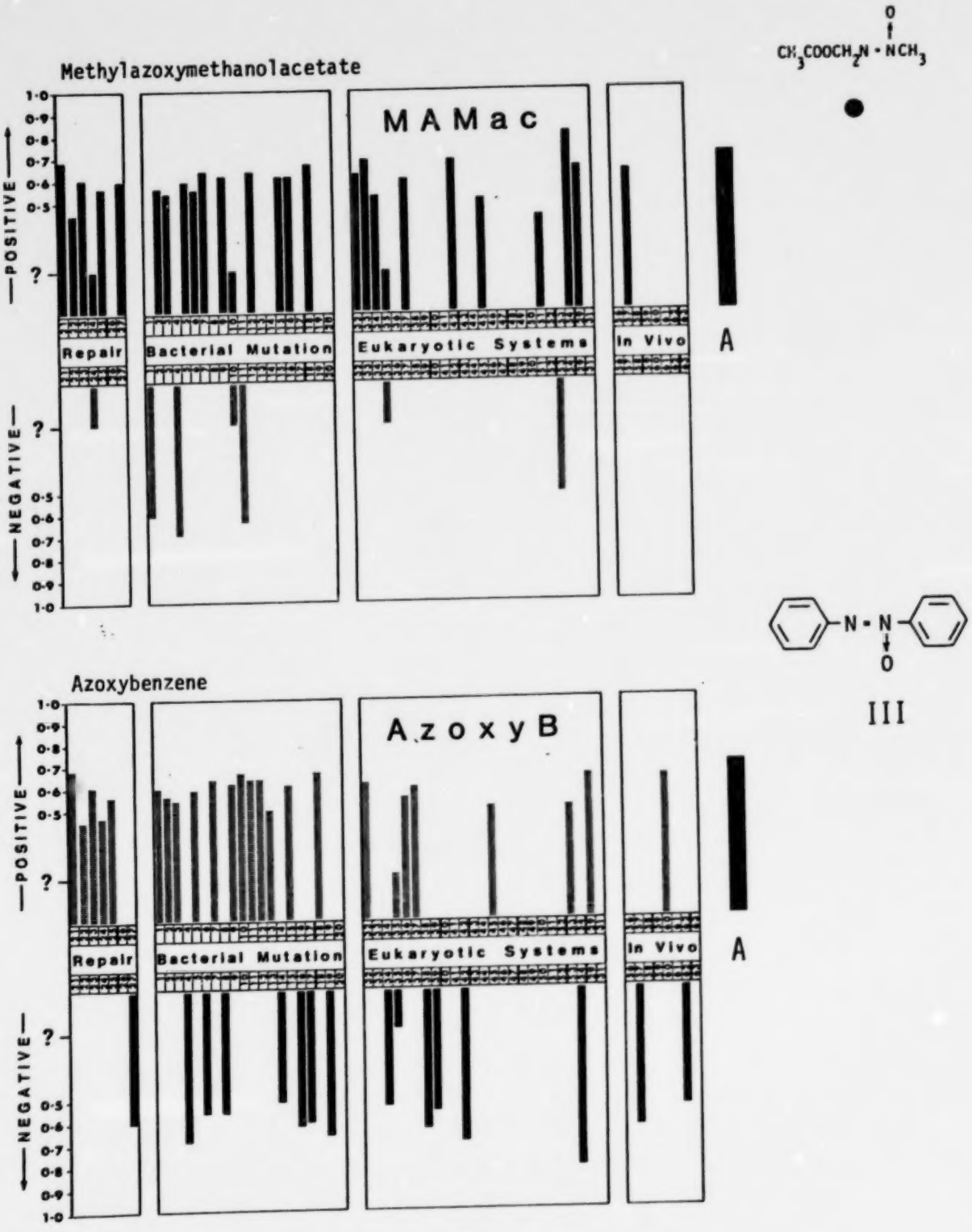
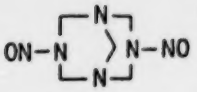

$NH_2COOC_2H_5$
Urethane
Urthne
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Cl
$NHCOOC_3H_7^i$
I
Isopropyl N(3-chlorophenyl)carbamate
IsoPC
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A

Figure 12.



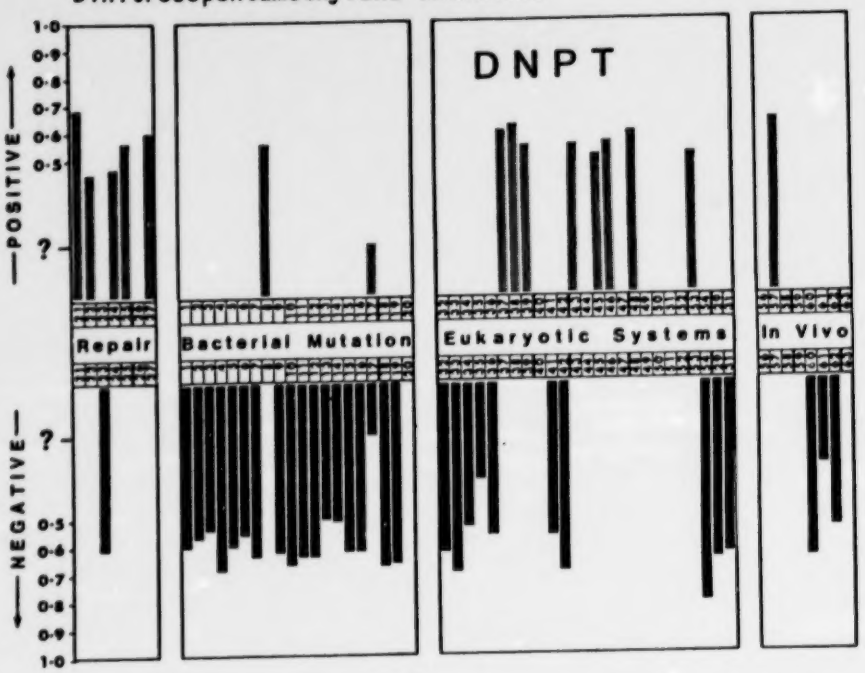
III

Figure 13.



III

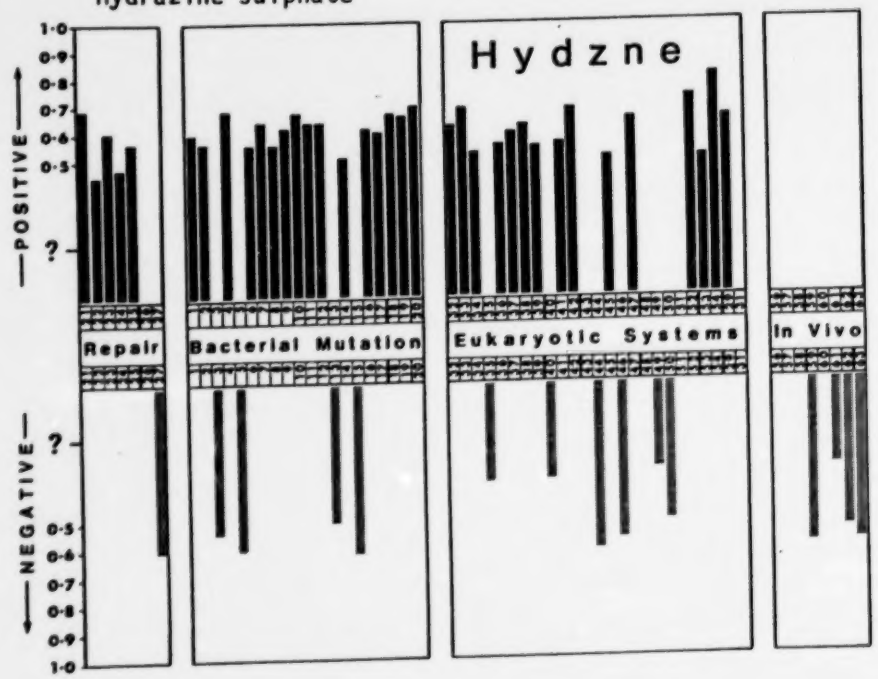
Dinitrosopentamethylene tetramine



A

$NH_2NH_2 \cdot H_2SO_4$

Hydrazine sulphate



A

Figure 14.

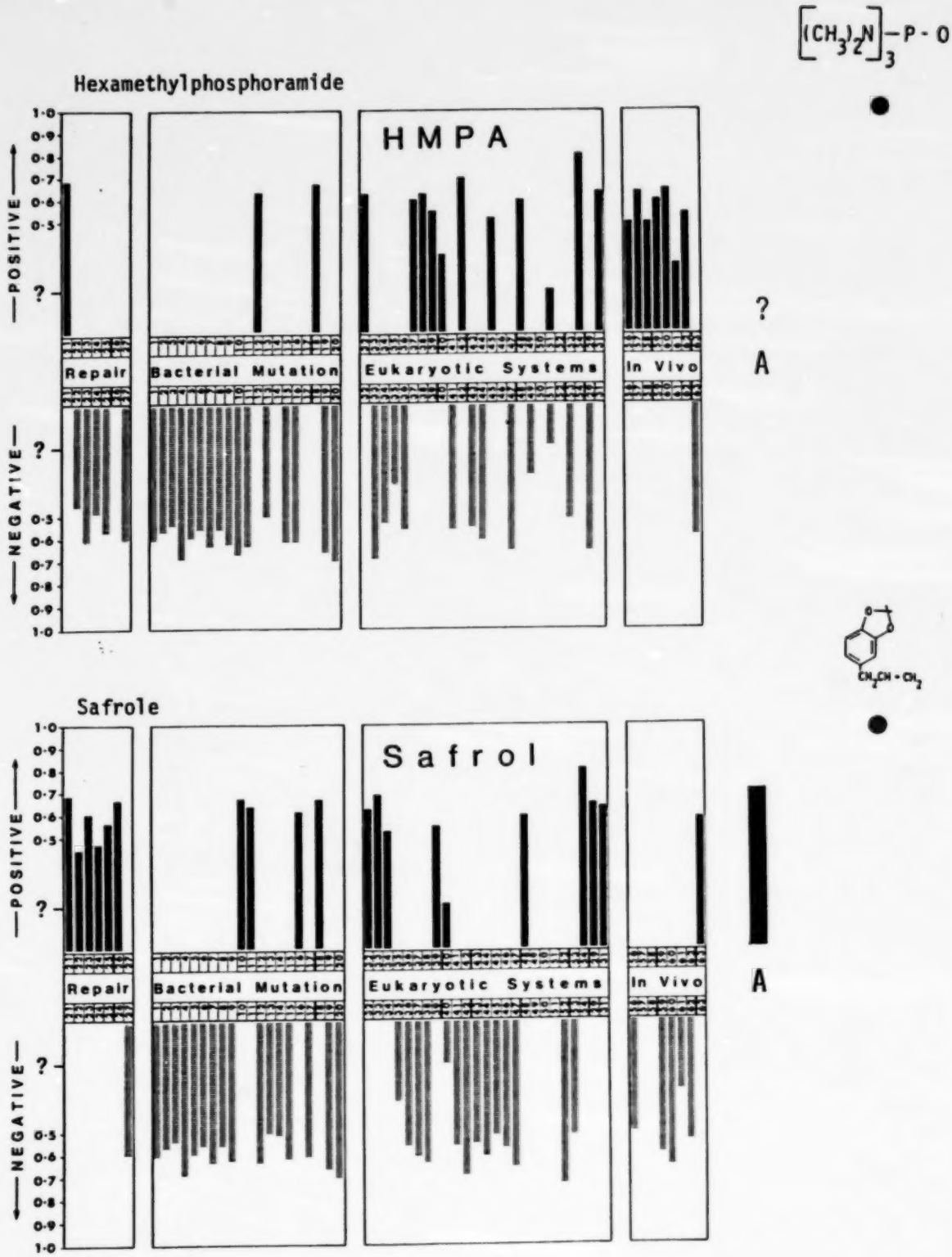

Hexamethylphosphoramide
HMPA
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
?
A
Safrole
Safrol
1·0
0·9
0·8
0·7
0·6
0·5
0·3

Figure 15.

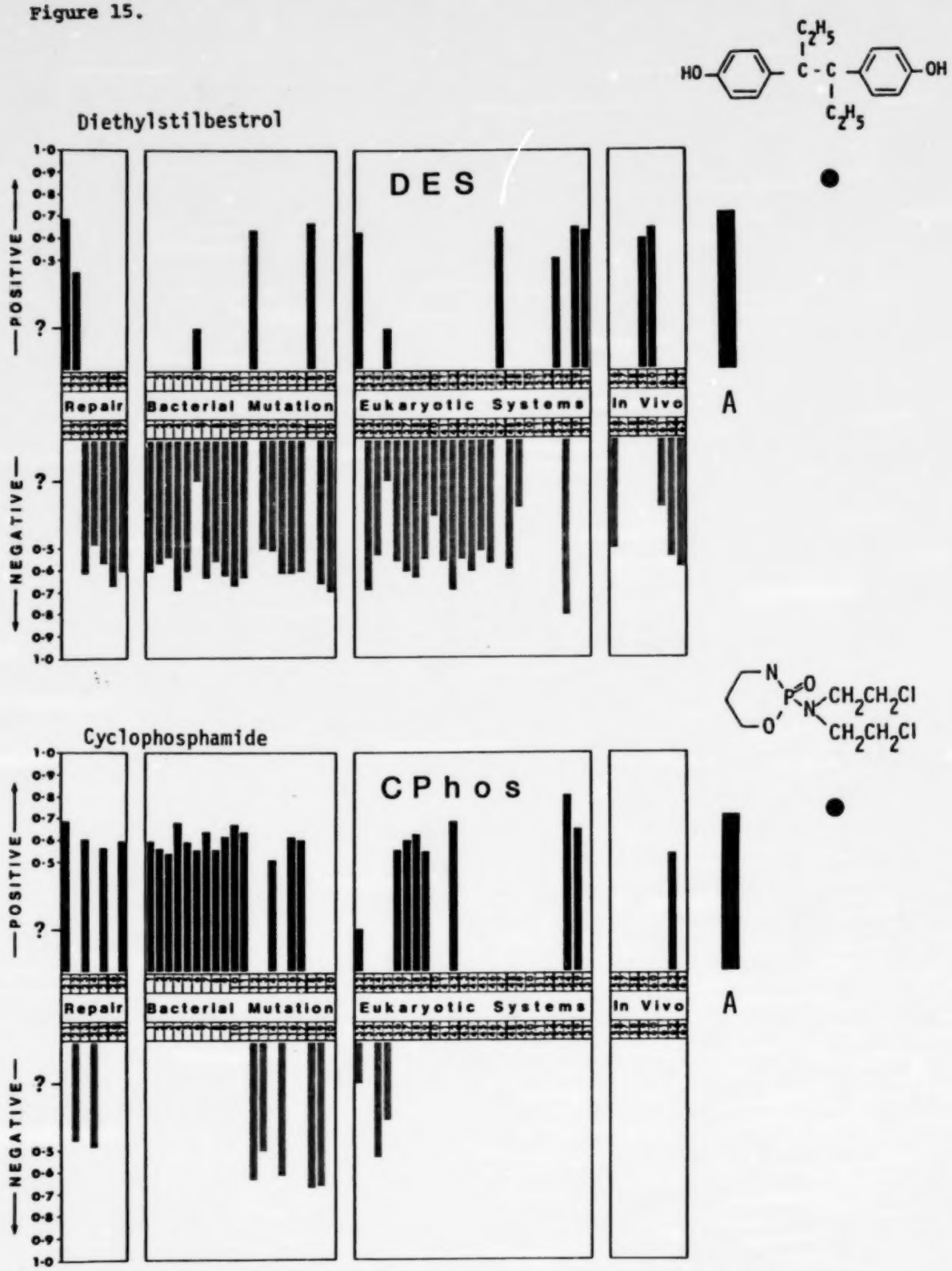

Diethylstilbestrol
DES
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Cyclophosphamide
CPhos
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A

Figure 16.

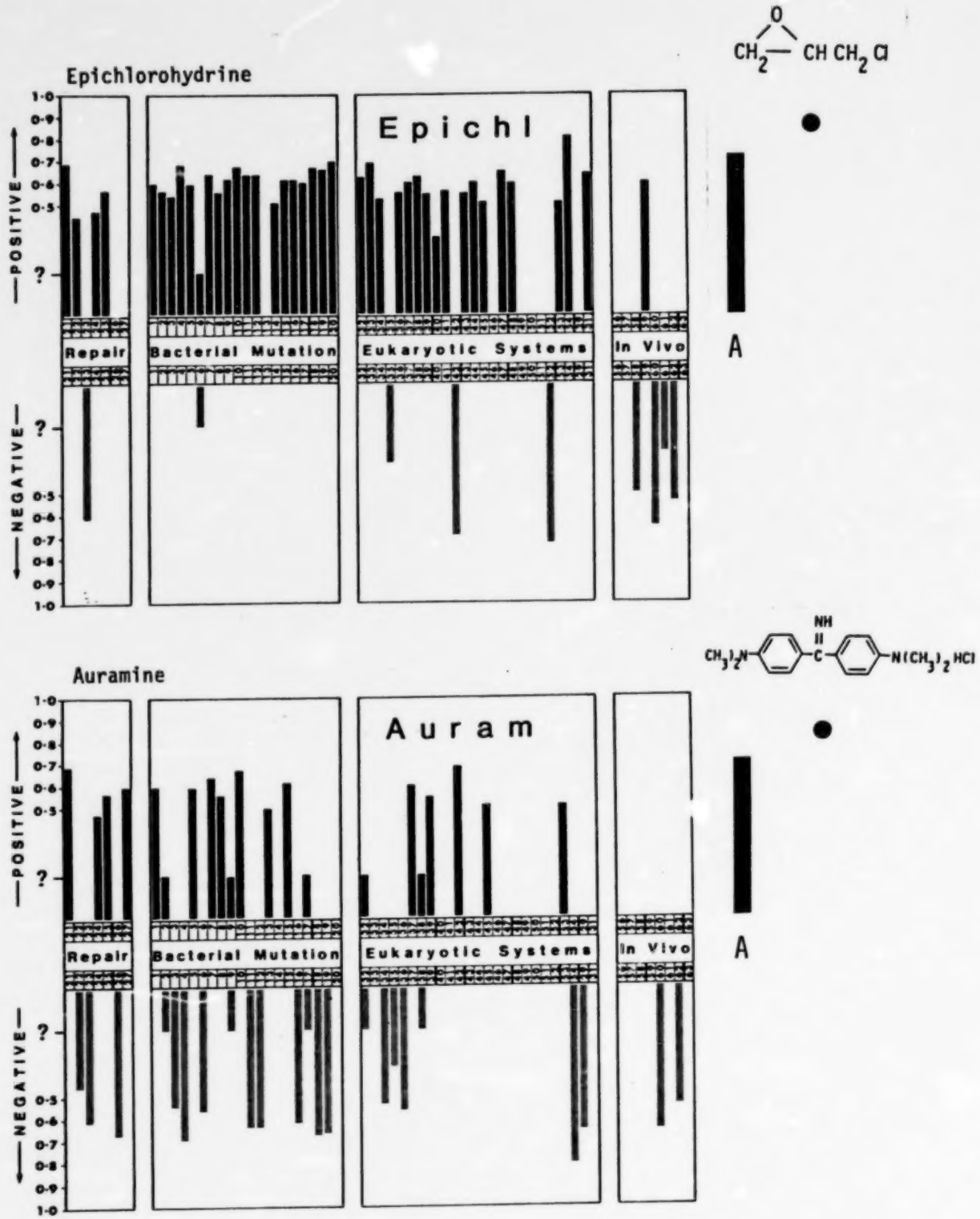

Epichlorohydrine
Epichl
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Auramine
Auram
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A

Figure 17.

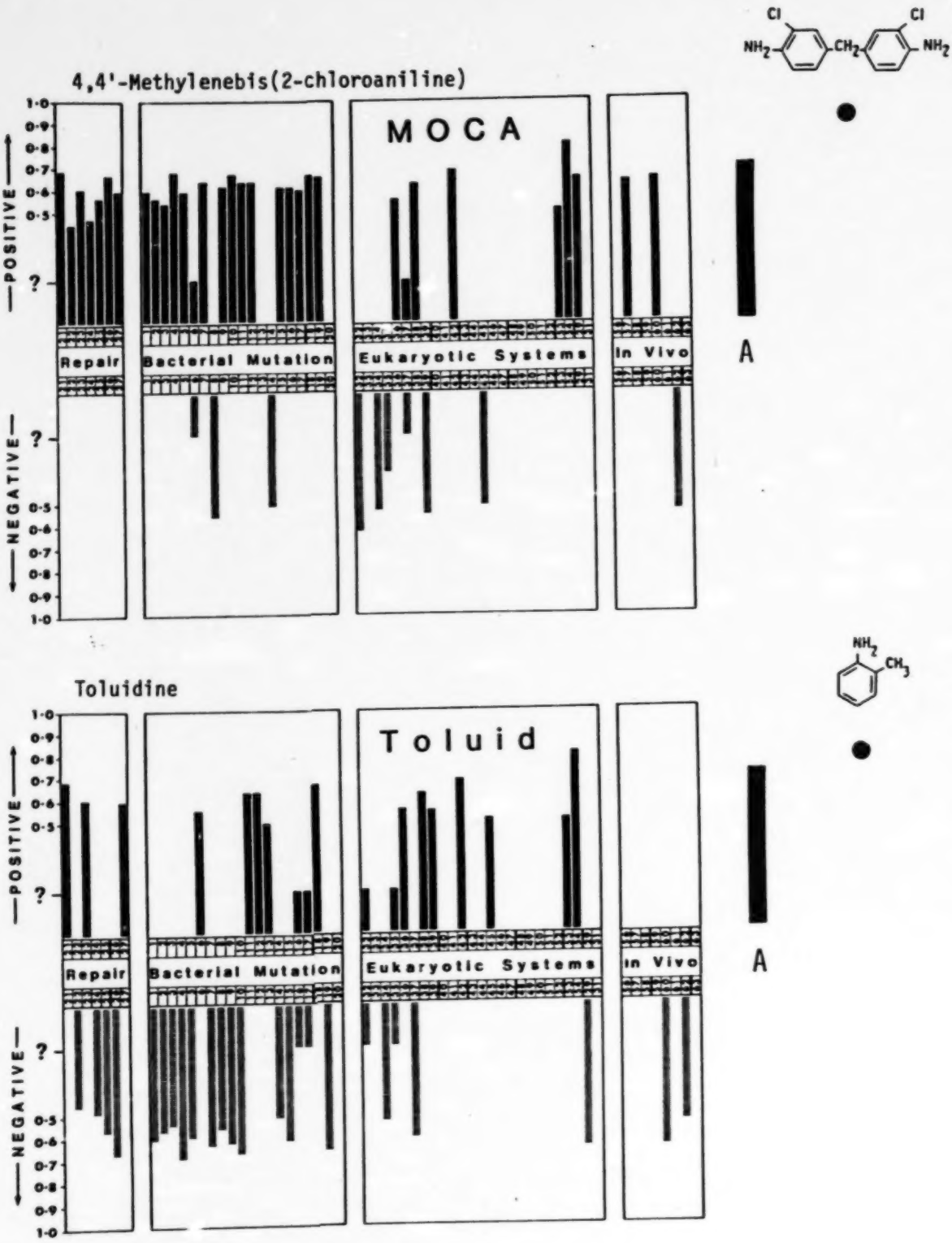

4,4'-Methylenebis(2-chloroaniline)
MOCA
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Toluidine
Toluid
in Vivo

Figure 18.

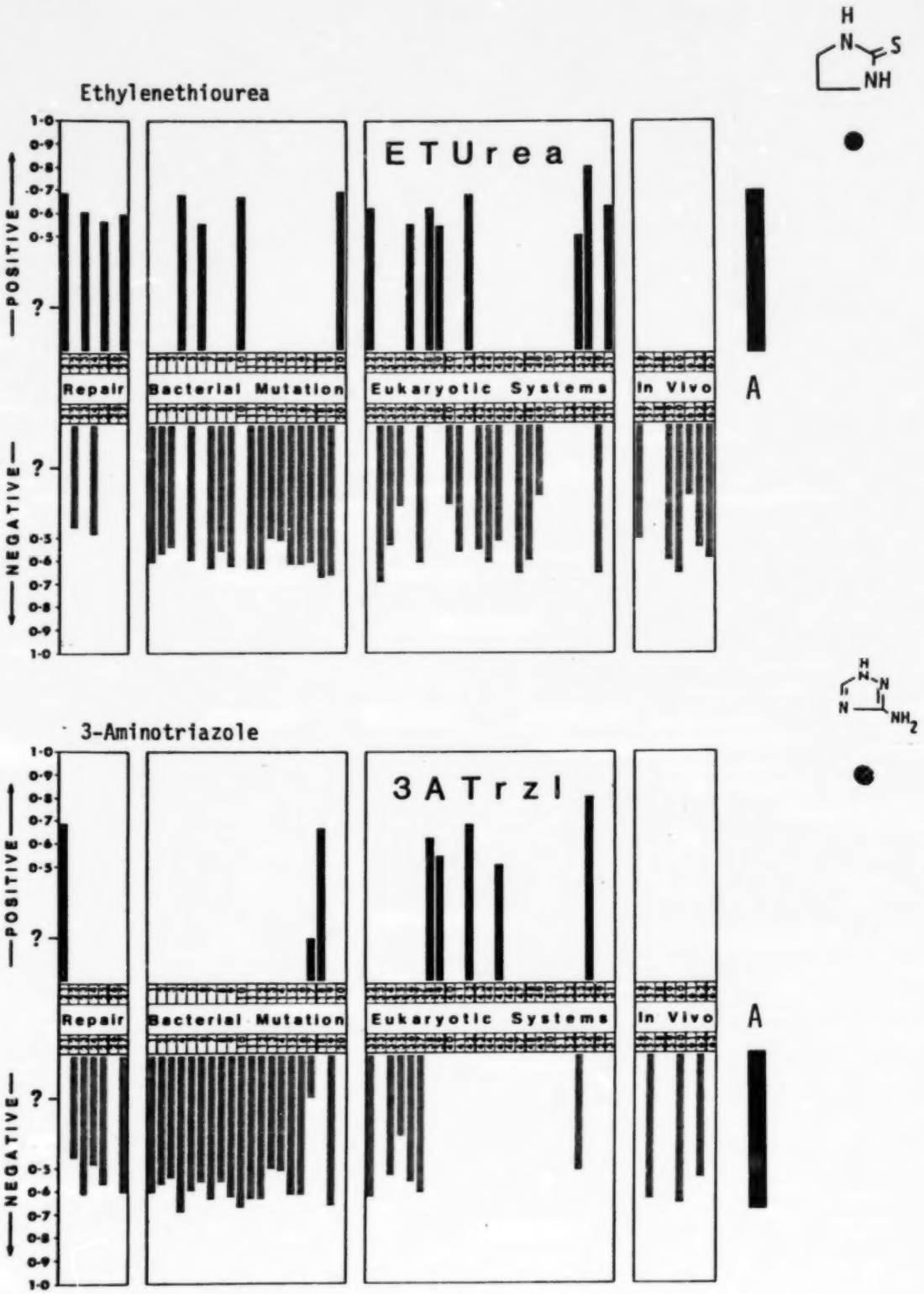

Ethylenethiourea
ETUrea
POSITIVE
NEGATIVE
?
1·0
0·9
0·8
0·7
0·6
0·5
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
3-Aminotriazole
3ATrzl

Figure 19.

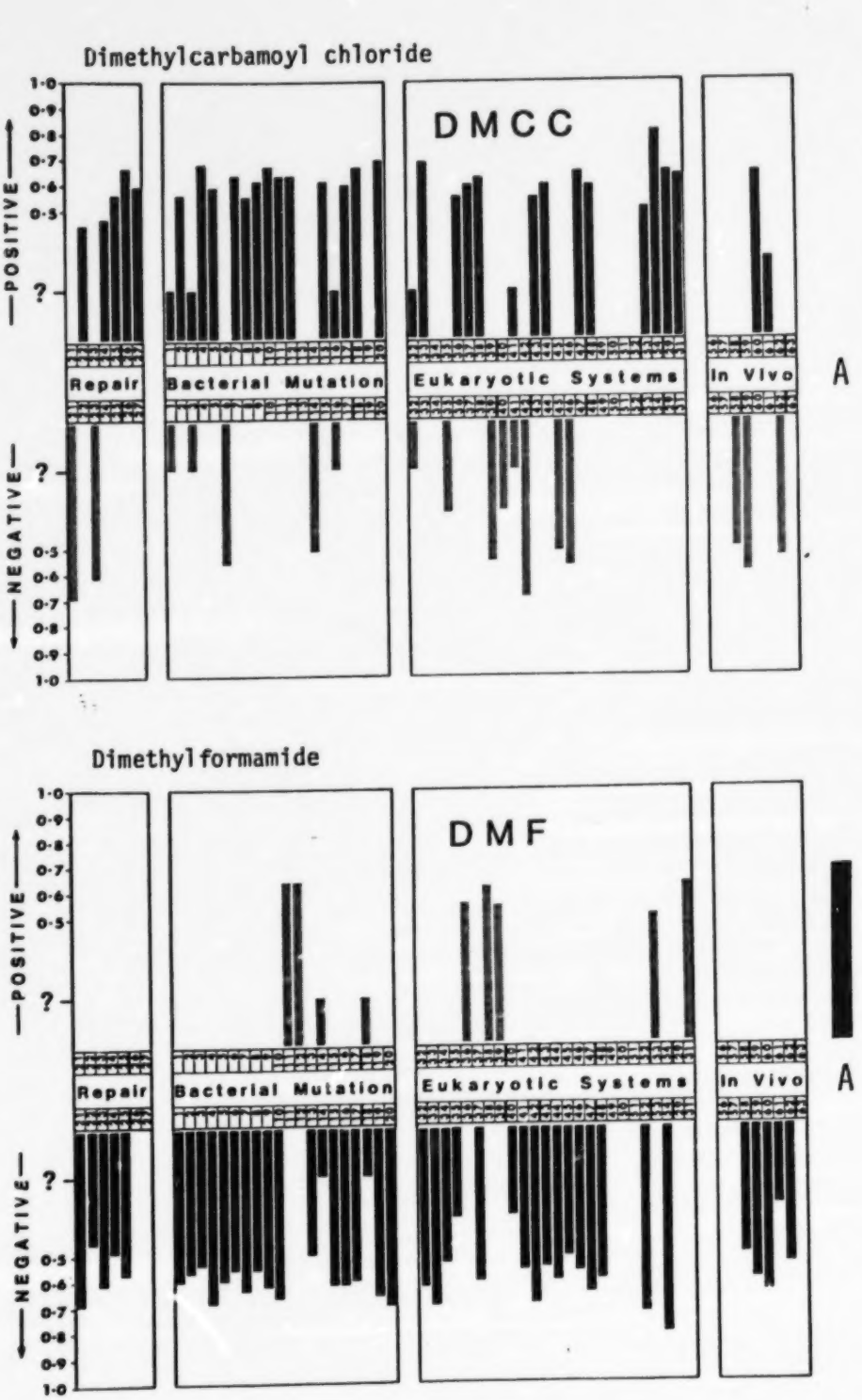

Dimethylcarbamoyl chloride
(CH3)2N.COCl
DMCC
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A
Dimethylformamide
HCON(CH3)2
II
DMF
POSITIVE
NEGATIVE
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
A

Figure 20.

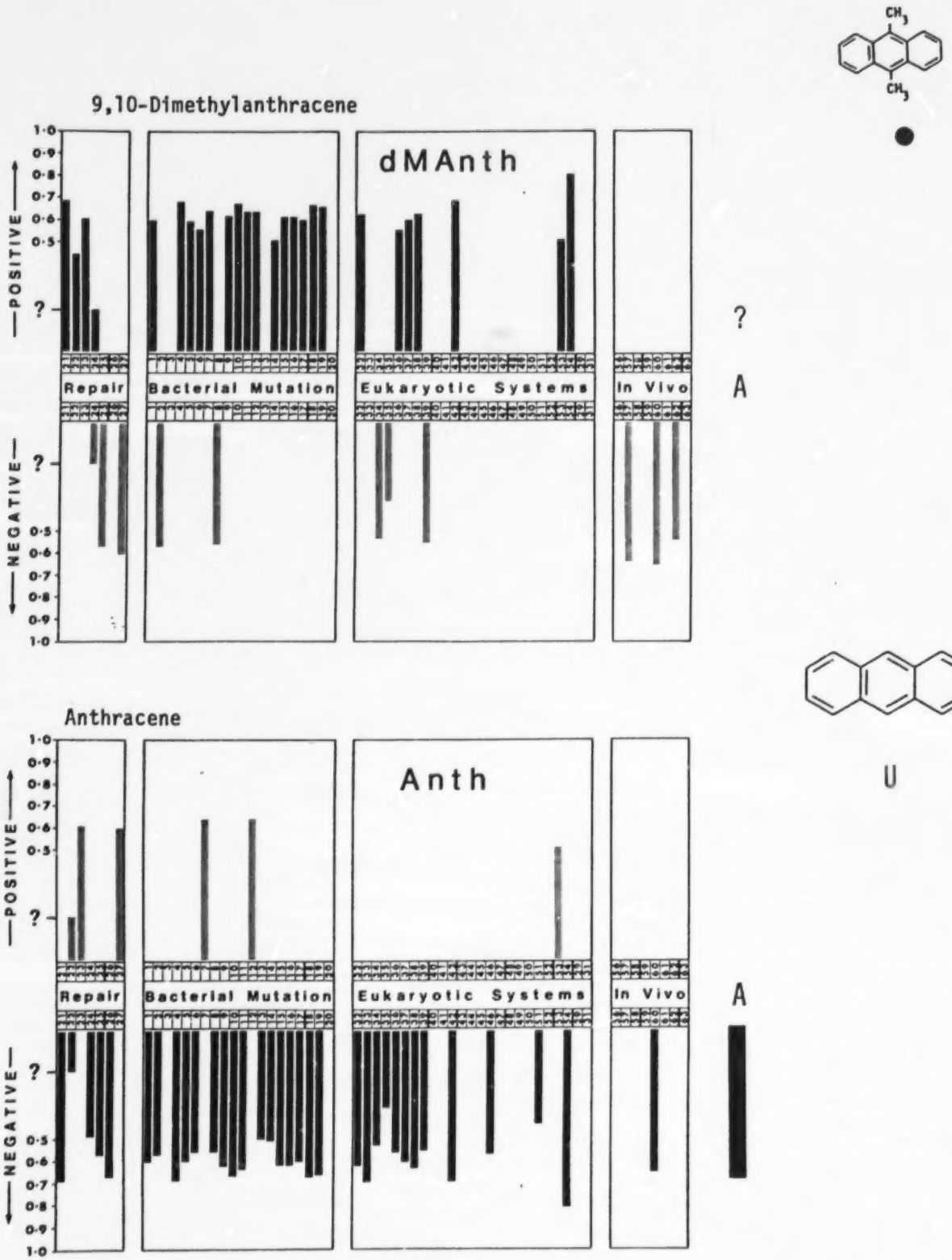
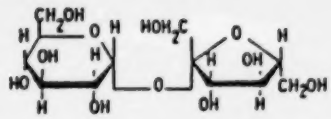
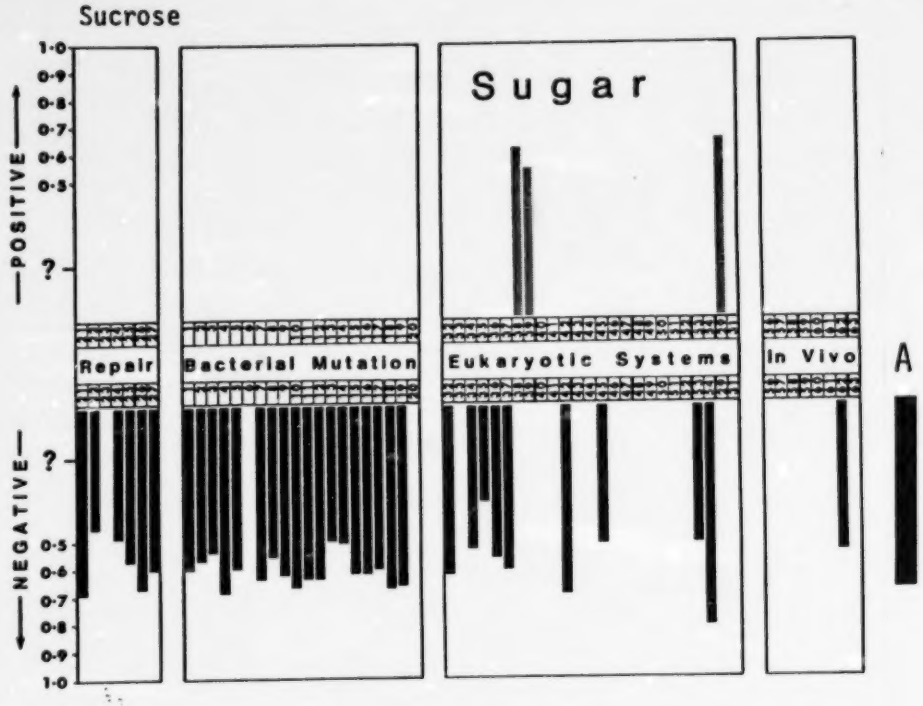
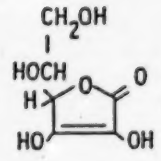

9,10-Dimethylanthracene
dMAnth
POSITIVE
NEGATIVE
1·0
0·9
0·8
0·7
0·6
0·5
?
Repair
Bacterial Mutation
Eukaryotic Systems
In Vivo
?
A
Anthracene
Anth
A
U

Figure 21.

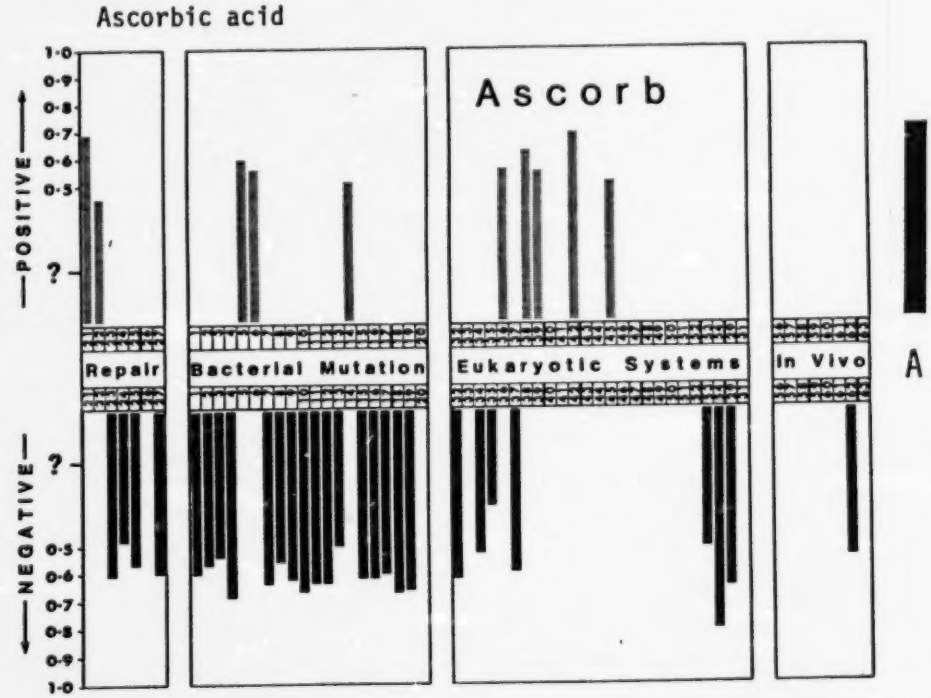


III





U



must be repeated (Fig. 2).

The results with the pair dimethylaminobenzene (carcinogen) and dimethylaminobenzene sulphonic acid (class II noncarcinogen) are quite controversial. Only 26/43 assays classified correctly the former chemical and according to 21/55 assays the noncarcinogen behaved as a carcinogen. Arabidopsis correctly identified the carcinogen but the noncarcinogenic analog has not been tested yet (Fig. 3).

Propiolactone (carcinogen) was properly classified by 38/41 of the tests, including Arabidopsis and butyrolactone (noncarcinogen) was correctly identified by nearly as successfully (35/43). Unfortunately, in the Arabidopsis assay the results were somewhat equivocal but most likely it was not mutagenic (Fig. 4).

Benzo(a)pyrene (carcinogen) was successfully identified by 47/57 assays, including Arabidopsis. Pyrene (unclassified noncarcinogen) behaved as a carcinogen in 21/55 of the assays. In Arabidopsis, except at the lowest concentration, it increased the number of mutations (Fig. 5).

Ethionine (carcinogen) was misclassified by the majority of assays in all four groups and only 16/57, including the Arabidopsis test, identified it correctly. The corresponding analog, methionine (U type noncarcinogen) was classified according to expectation by over 80% of the tests. Methionine was not mutagenic in the Arabidopsis assay (Fig. 6).

Chloroform (carcinogen) was properly identified by less than 20% (8/41) of the assays. This chemical was found to be mutagenic, however, by the Arabidopsis test. Trichloroethane (the II noncarcinogen) was classified according to the predictions by 33/41 of the test. The Arabidopsis test, along with seven other systems, classified it mutagenic, however, contrary

to the expectation (Fig. 7).

2-Acetylaminofluorene (carcinogen) and 4-acetylaminofluorene (type U noncarcingoen) both were classified by the majority of the tests as carcinogens; the scores were 37/43 (86%) and 30/41 (73%), respectively. Both compounds were mutagenic for Arabidopsis (Fig. 8).

2-Naphtylamine (carcinogen) and 1-naphtylamine (type II noncarcinogen) both appeared carcinogens as expected in most of the tests by the scores of 43/57 and 34/58, respectively. The results with Arabidopsis in case of 2-naphtylamine were inconclusive but 1-naphtylamine was mutagenic (Fig. 9).

Nitrosomorpholine (carcinogen) and diphenylnitrosamine (type II noncarcinogen) gave rather mixed responses because in 60% (30/50) and 34% (17/50) of the tests, including the Arabidopsis assay, both seemed to respond as carcinogens are expected (Fig. 10).

Urethane (carcinogen) was identified poorly in as much as only 18/47 tests showed positive results. In Arabidopsis urethane was mutagenic, confirming earlier findings by Roebbelen (1962) and Müller (1967). Isopropyl chlorophenyl urethane (class I noncarcinogen) was classified as noncarcinogen by 29/43 of the tests. This was a poor showing for a type I noncarcinogen. In the Arabidopsis assay both compounds were mutagenic (Fig. 11).

Methylazoxymethanolacetate (carcinogen) and azoxybenzene (class III noncarcinogen) both were classified as carcinogens by 81% (29/36) and 62% (26/42) of the tests, respectively. In the Arabidopsis assays both compounds appeared mutagenic (Fig. 12).

Dinitrosopentamethylene tetramine (type III noncarcinogen) was classified as a noncarcinogen by 30/48 of the tests. This compound was not tested with Arabidopsis. Hydrazine sulphate (carcinogen) was correctly

identified by 37/52 fraction of the assays, including Arabidopsis (Fig. 13).

Hexamethylphosphoramide (carcinogen) and safrole (carcinogen) both had rather poor showing in the tests; 21/55 and 21/57 minority of the tests identified them according to expectation. The former gave inconclusive results with Arabidopsis, the latter was mutagenic (Fig. 14).

Diethylstilbestrol and cyclophosphamide (carcinogens) evoked very different responses. The former was classified correctly in only about a fourth (14/58) of the assays whereas the latter gave positive results in about three fourths (28/38) of the systems. Arabidopsis identified both compounds as expected on the basis of their carcinogenicity (Fig. 15).

Epichlorohydrine (carcinogen) and auramine (carcinogen) were correctly identified by 43/52 and 22/45 of the tests, respectively. For Arabidopsis both compounds were mutagenic (Fig. 16).

Methylenebis(2-choloroaniline) and toluidine (both are carcinogens) were identified with unequal efficiency. The success was 33/43 and 20/45, respectively. Both compounds were mutagenic in the Arabidopsis assays (Fig. 17).

Ethylenethiourea and aminotriazole (carcinogens) were correctly identified by only a few systems. The overall efficiency of the success was 17/54 and 8/41, respectively. Ethylenethiourea was mutagenic for Arabidopsis but aminotriazole was not (Fig. 18).

Dimethylcarbamoyl chloride (carcinogen) was properly classified by 37/55 fraction of the tests. This compound has not been tested yet with Arabidopsis. Dimethylformamide (type II noncarcinogen) appeared to be a noncarcinogen by 34/44 of the assays but it was mutagenic for Arabidopsis (Fig. 19).

Dimethylanthracene (carcinogen) was identified as expected by 26/37 of the tests. The results with Arabidopsis were inconclusive. Anthracene was not mutagenic for Arabidopsis (F.g 20).

Sucrose (class III noncarcinogen) was classified according to expectation 32/35 of the assays; it was not mutagenic in the Arabidopsis tests. Ascorbic acid (type U noncarcinogen) was classified as a noncarcinogen by about 72% of the tests. In one experiment involving 128,000 genomes of ascorbic acid-treated and negative control, the difference in the frequency of seedling mutations was significant at the 0.05 level of probability, calculated with the aid of the table of Kastenbaum and Bowman (1970). In the seven repeated Arabidopsis assays, among 17 separate observations 15 indicated an excess of the mutational events in the population exposed to ascorbate compared to the negative control. The frequency of the positive observations 15/17 = 0.88235 exceeds the confidence limits at 99% probability that the positive values scored would be random fluctuations. Actually, the upper limits of confidence at the 99.9% probability would be 0.5 ± 0.39897; thus, the Arabidopsis data observed in the ascorbate experiments almost meet the 0.001 level of significance (Fig. 21 and Table 5).

The data shown in Figures 1 to 21 can be used for estimating the accuracy of the Arabidopsis assay in comparison to the groups of tests, namely prokaryotic repair, bacterial mutation, eukaryotic assays and in vivo tests. Also, we can determine the accuracy of identification of each chemical by the various groups of assay systems (Table 6).

In Table 6 the information is grouped according to the five types of assays conducted. Within each group the data of individual experimenters or tests are lumped. At the bottom of the table the accuracy of these

Table 5.

EFFECT OF ASCORBIC ACID ON MUTATION FREQUENCY IN ARABIDOPSIS

Concentration of Ascorbic Acid	Frequency of Plants With Pale Embryos (%), Control Values Subtracted						Frequency of Seedling Mutations Per Genome	
							Control	Treated
Exp.	I	II	III	IV	V	VI		
0.05	7.8	2.0	1.5	4.4	2.8	-	-	-
0.10	4.4	3.0	-	-	-	-	-	-
0.20	6.2	-	0	11.3	4.5	6.5	1.6×10^{-4}*	3.4×10^{-4}*
0.50	0	-	-	-	-	-	-	-
1.00	6.6	-	3.5	-	-	-	-	-
2.00	4.2	-	-	-	-	-	-	-

* The difference exceeds the 5% confidence limits; both control and treated populations involved 124,000 genomes

systems is summarized. The overall success in correct identification of all the systems was only 60.8%. The relative ranking is Arabidopsis assay (68.8%), bacterial mutation (62.1%), eukaryotic systems (59.5%), repair assays in prokaryotes (58.3%), and in vivo tests in mammals and insects (57.3%). It is somewhat disappointing that the in vivo tests (with the exception of Arabidopsis) showed the least reliable performance.

Data of Table 6 (right-most column) express quantitatively the general detectability of carcinogens (and identification of noncarcinogens) by the short-term bioassays involved in this study. These figures may be used for reclassification of the compounds. Obviously, if a "noncarcinogen" shows

Table 6. Accuracy of the five assay systems and of the identification of 42 chemicals. Accuracy is defined as the percent of correct identification according to the originally predicted carcinogenicity. The signs • and – indicate carcinogenicity and noncarcinogenicity, respectively, NT = not tested, ? = data inconclusive.

Chemicals		Accuracy					
		Repair Tests	Bacterial Mutation	Eukaryotic Systems	In Vivo Tests	ARABIDOPSIS ASSAYS	Total
Nitroquinoline-N-oxide	•	100	100	91	20	NT	89
Methylnitroquinoline-N-oxide	–	0	0	5	60	NT	8
Benzidine	•	57	85	68	40	100	68
Tetramethylbenzidine	–	71	95	88	88	?	88
Dimethylaminobenzene	•	0	45	64	40	100	48
Dimethylaminobenzene-sulphonic acid-Na	–	71	45	65	100	NT	62
β-Propiolactone	•	100	100	92	33	100	93
γ-Butyrolactone	–	67	90	71	100	?	81
Benzo(a)pyrene	•	71	100	71	83	100	83
Pyrene	–	75	57	53	100	0	62
Ethionine	•	29	14	35	40	100	28
Methionine	–	67	90	76	100	100	81
Chloroform	•	17	11	31	0	100	20
Trichloroethane	–	83	94	67	100	0	81
2-Acetylaminofluorene	•	71	100	73	100	100	86
4-Acetylaminofluorene	–	33	0	50	100	0	27
2-Naphthylamine	•	71	95	75	29	?	75
1-Naphthylamine	–	50	30	36	78	0	41
Nitrosomorpholine	•	43	70	56	50	100	60
Diphenylnitrosamine	–	57	70	65	80	0	66
Urethane	•	38	26	41	100	100	38
Isopropyl N(3-chlorophenyl carbamate)	–	50	100	38	100	0	67
Methylazoxymethanol-acetate	•	86	73	83	100	100	81
Azoxybenzene	–	17	37	46	67	0	38
Dinitrosopentamethylene tetramine	–	17	90	56	75	?	67
Hydrazine sulphate	•	83	80	21	0	100	71
Hexamethylphosphoramide	•	17	11	48	88	?	38
Safrole	•	86	20	39	20	100	38
Diethylstilbestrol	•	29	14	26	33	100	24
Cyclophosphamide	•	67	74	73	100	100	74
Epichlorohydrine	•	80	95	20	20	100	82
Auramine	•	57	48	50	0	100	49
Methylenebis(2-chloroaniline)	•	100	84	54	66	100	77
Toluidine	•	43	33	64	0	100	44
Ethylenethiourea	•	67	20	38	0	100	32
Aminotriazole	•	17	10	46	0	0	20
Dimethylcarbamoyl chloride	•	71	76	64	40	?	67
Dimethylformamide	–	100	82	76	100	0	82
Dimethylanthracene	•	57	88	70	0	?	70
Anthracene	–	63	89	92	100	100	85
Sucrose	–	100	100	75	100	100	92
Ascorbate	–	67	84	58	100	0	72
Accuracy of the Systems		58.3	62.1	59.5	57.3	68.8	60.8

positive response in most of the assays, one must regard with some suspicion the validity of the carcinogenicity information. Similarly if a "carcinogen" is undetectable by the majority of the short-term assays, one may wish to check the reliability of the information provided by the direct rodent tests.

It is conceivable that the direct carcinogenicity tests have missed some of the less effective carcinogens because the animal tests frequently involve smaller population than the short-term assays. Table 7 shows the performance of the five types of short-term assays in cases when the original carcinogenicity information (obtained from direct cancer tests) is used for the calculation of accuracy. These indicators of accuracy compared then with the data calculated after some reclassification. As we can see from Table 6, methylnitroquinoline-N-oxide, 1-naphthylamine, 4-acetylaminofluorene, diphenylnitrosamine and azoxybenzene displayed the accuracy figures 8, 41, 27, 66, and 38, respectively. Also, these compounds were originally classified as ambiguous noncarcinogens; their rating in Table 1 is III, II, U, II amd III. respectively.

This reclassification leads to a substantial improvement in the accuracy scores of the Arabidopsis assays as well three other types of assays but lowered the accuracy estimates of the other in vivo systems (Table 7).

Table 7. Performance of the five assay systems after reclassifying methylnitroquinoline, 1-naphthylamine, 4-acetylaminofluorene, diphenylnitrosamine and azoxybenzene as carcinogens. Figures are accuracy estimates (%).

	Repair Tests	Bacterial Mutation	Eukaryotic Systems	In Vivo Tests	ARABIDOPSIS ASSAYS
Original Classification	58.3	62.1	59.5	57.3	68.8
Reclassification Scores	65.9	67.2	63.4	51.5	81.3

The accuracy estimates may be high either because an assay correctly identifies the true carcinogens and the genuine noncarcinogens, or the figures may be high because either the carcinogens or the noncarcinogens were misclassified originally and the short-term test merely confirmed the errors. Ashby (1981) considered eight compounds involved in this project (listed in Table 8) as the most likely true noncarcinogens.

Table 8. Response of the Arabidopsis assay to eight compounds deemed to be true noncarcinogens according to the revision by Ashby (1981). The signs •, - and ? indicate mutagenic, nonmutagenic and inconclusive results, respectively.

Chemicals	Classification in Arabidopsis
Isopropyl N(3-chlorophenylcarbamate)	•
Methionine	-
Ascorbic Acid	•
Sucrose	-
Dimethyformamide	•
Tetramethylbenzidine	?
γ-Butyrolactone	?
Anthracene	-

Three of this eight compounds appeared mutagenic, however, in Arabidopsis. Extensive analysis on the mutagenicity of these noncarcinogens was carried out so far only with ascorbate. Repeated tests were consistent (Table 5), thus we must consider ascorbic acid as a mutagen. Actually, ten of the tests conducted in other laboratories involved in this project have made similar observations in other systems. Also, some independent mutagenic assays in Salmonella (Stich et al. 1976), chromosomal aberrations in Chinese hamster ovary cells (Stich et al. 1980), sister chromatid exchange (Galloway & Painter 1979), locus-specific mutations in mouse lymphoma cells (Amacher &

Paillet 1981) as well as bacteriophage or DNA inactivation (Richter & Loewen 1982, Mita et al. 1982) have been observed as a consequence of ascorbate treatment. Some of the other noncarcinogens require further analysis by Arabidopsis.

One remarkable feature of the Arabidopsis assay was revealed by these tests. All assays identified,with over 50% sensitivity,14 of the 25 carcinogens involved in this study; 11 of the carcinogens proved to be very difficult to identify with other assays, especially with bacterial mutation tests (e.g. Ames test with S-9). The Arabidopsis test classified as mutagens 10/14 carcinognes which can be reasonably well identified with bacteria. One compound could not have been tested yet and three gave inconclusive results and require retesting (Table 9).

From the "problem carcinogens" for the bacterial systems (listed below the double line) 9/11 was mutagenic for Arabidopsis and only 1/11 appeared nonmutagenic; another could not be classified with certainty (Table 9). This group of 11 carcinogens cannot be identified in a satisfactory manner by the bacterial mutation assays and turned out to be very ambiguous in all other systems except Arabidopsis.

Carcinogens can be classified into two broad groups: genotoxic and epigenetic agents (Weisburger & Williams 1980). The second group include agents which act mainly by physical rather than chemical means or their action is rather indirect and concerns hormones, the immunological defense system or enhanced by various other ways the action of genotoxic agents without being carcinogens themselves.

The genotoxic agents may be carcinogens which directly affect the DNA or they require chemical transformation by enzymes (activation) before becoming ultimate mutagens or carcinogens. Some heavy metals also may

Table 9. A comparison of the sensitivity of all assays combined, of the bacterial mutation tests and of the Arabidopsis assays. Sensitivity is defined as the fraction of correct identification of carcinogens relative to all carcinogens tested. For this table only 25 carcinogens were chosen. The decimal fractions are expressed as percent in the body of the table. The signs •, - and ? indicate mutagenicity, nonmutagenicity and inconclusive results, respectively; NT = not tested.

Carcinogens	Sensitivity		
	All Assays	Bacterial Mutation	ARABIDOPSIS
β-Propiolactone	93	100	•
4-Nitroquinoline-N-oxide	89	100	NT
2-Acetylaminofluorene	86	100	•
Benzo(a)pyrene	83	100	•
Epichlorohydrin	82	95	•
Methylazoxymethanolacetate	81	73	•
Methylenebis(2-chloroaniline)	77	84	•
2-Naphthylamine	75	95	?
Cyclophosphamide	74	74	•
Hydrazine sulphate	71	80	•
Dimethylanthracene	70	88	?
Benzidine	68	85	•
Dimethylcarbamoyl chloride	67	76	?
Nitrosomorpholine	60	70	•
Auramine	49	48	•
Dimethylaminobenzene	48	45	•
O-Toluidine	44	33	•
Hexamethylphosphoramide	38	11	?
Safrole	38	20	•
Urethane	38	26	•
Ethylenethiourea	32	20	•
Ethionine	28	14	•
Diethylstilbestrol	24	14	•
Chloroform	20	11	•
Aminotriazole	20	10	-

directly become carcinogens by interfering with the normal course of DNA replication.

The direct acting or primary carcinogens should be carcinogenic (mutagenic) in all types of cells unless the organism possesses a detoxification mechanism. The indirect acting chemicals (procarcinogens or secondary carcinogens) may show great variation in their effect in various organisms or types of cells, depending on the presence of an enzyme system capable of converting them into an active (ultimate) carcinogen.

The procarcinogens actually pose greater health hazards to human populations than any other types of carcinogens because of the difficulties involved in their detection. These procarcinogens may not be detected with equal facility in the various direct mammalian carcinogen assays and are even more likely to escape identification by the short-term assays.

The poor performance of the Ames/S-9 Salmonella test with the compounds listed below the doulbe line in Table 9, seems to indicate that these 11 chemicals are not activated effectively by the microsomal fraction used. Although it is not well known how these or most other chemicals initiate oncogenic transformation in mammals, and it seems certain that those chemicals which are inactive in one or more systems but carcinogenic in others, are procarcinogens requiring specific activation.

On the basis of information provided by a number of microbial experiments using or not using the S-9 fraction (de Serres & Ashby 1981), we classified these 42 chemicals regarding their presumable need for activation (Table 10). Of the 15 chemicals that most likely require activation 12 was mutagenic for Arabidopsis, 2 gave inconclusive results and 1 had not been tested yet. Accordingly, Arabidopsis seeds seem to have all the activating enzymes provided by the mammalian liver fraction used in the microbial assays. Also,

Table 10. Determination of the requirement for activation of the test chemicals on the basis of the results with (S+) and without (S-) the liver fraction. Data of the microbial tests from de Serres & Ashby, 1981.

Code no.	Original Classification	Chemical	Arabidopsis Assays	KADA B. subtilis, repair S-	KADA B. subtilis, repair S+	BAKER & BONIN Salmonella, Ames test S-	BAKER & BONIN Salmonella, Ames test S+	BROOKS & DEAN Salmonella, Ames test S-	BROOKS & DEAN Salmonella, Ames test S+	RICHOLD & JONES Salmonella, Ames test S-	RICHOLD & JONES Salmonella, Ames test S+	ROWLAND & SEVERN Salmonella, Ames test S-	ROWLAND & SEVERN Salmonella, Ames test S+	MEHTA & von BORSTEL Saccharomyces, reversion S-	MEHTA & von BORSTEL Saccharomyces, reversion S+	LOPRIENO Schizosaccharomyces forward S-	LOPRIENO Schizosaccharomyces forward S+	JAGGENATH et al. Saccharomyces, conversion S-	JAGGENATH et al. Saccharomyces, conversion S+	*Presumably, activation is required*
1	+	Butter yellow	+	-	-	-	+	+	+	-	-	-	-	?	±	-	-	?	?	✓
2	III	Tetramethylbenzidine	?	+	-	-	-	-	-	-	-	-	-	?	-	-	-	-	-	
3	U	4-Acetylaminofluorene	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	+	✓
4	II	1-Napthylamine	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	✓
5	U	Pyrene	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	?
6	+	Chloroform	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	?
7	+	Diethylstilboestrol	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	
8	+	4NQO	?	+	-	+	-	-	-	-	+	-	-	+	○	-	+	+	+	✓
9	+	2-Napthylamine	?	+	+	-	+	+	+	-	+	-	+	?	+	-	-	-	-	✓
10	II	4-Dimethylaminoazobenzene-4-sulph. acid	○	-	-	-	+	-	-	-	+	-	+	-	?	+	+	-	-	✓
11	+	Benzidine	+	+	+	-	+	+	+	-	+	-	+	-	-	-	-	-	-	✓
12	+	2-AAF	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	✓
13	+	Benzo(a)pyrene	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	+	✓
14	+	Hydrazine SO4	+	+	+	+	+	-	+	+	+	+	+	+	○	+	+	-	?	?
15	III	3-Met-4NQO	○	+	+	+	+	+	+	+	+	-	+	+	○	-	+	+	+	?
16	+	Hexa-met-phosphoramide	?	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?
17	+	Diphenylnitrosamine	+	+	-	+	-	-	-	-	-	-	-	?	+	-	-	-	-	
18	U	Anthracene	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	-	-	
19	III	Dinitrosopentamethylene tetramine	○	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
20	+	Ethylenethiourea	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	
21	III	γ-Butyrolactone	?	-	+	-	-	-	-	-	-	-	-	-	?	-	-	-	-	?
22	U	Methionine	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	-	-	
23	+	Safrole	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	✓
24	II	Dimethylformamide	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
25	+	DL-Ethionine	+	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	✓
26	+	Epichlorhydrin	+	+	-	+	+	+	+	+	+	+	+	+	○	+	+	-	-	
27	+	N-Nitromorpholine	+	+	-	-	+	-	+	○	+	-	+	+	-	+	+	-	-	✓
28	I	Isoprophyl N (3-chlorophenyl) carbamate	+	-	-	-	-	-	-	-	-	-	-	-	○	○	○	-	-	
29	+	β-propiolactone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
30	II	1,1,1-trichloroethane	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
31	+	Urethane	+	+	+	-	-	-	-	-	+	-	-	-	?	-	-	-	-	
32	+	Dimethylcarbamoyl chloride	+	-	+	?	?	+	+	+	+	+	+	?	-	+	+	-	-	
33	+	Azoxybenzene	+	-	+	-	+	-	+	-	-	-	+	?	-	○	○	-	?	✓
34	+	Cyclophosphamide	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	
35	+	3-Aminotriazole	-	-	+	-	-	-	-	-	-	-	-	-	-	○	○	-	-	?
36	+	MOCA	+	+	+	-	+	-	+	-	-	-	+	-	-	○	○	-	-	✓
37	+	Methylazoxymethanolacetate	+	+	+	-	-	+	+	○	○	-	+	+	○	○	○	-	?	
38	III	Sucrose	-	-	-	-	-	-	-	-	-	○	○	-	?	○	○	-	-	
39	+	Dimethylanthracene	?	-	+	-	+	+	+	+	+	-	+	-	-	○	○	-	-	✓
40	U	Ascorbic acid	+	-	+	-	-	-	-	-	-	-	-	-	-	○	○	-	-	?
41	+	o-Toluidine HCl	+	+	+	-	-	-	-	-	-	-	-	-	-	○	○	-	-	
42	+	Auramine	+	-	+	-	+	-	-	-	-	-	?	-	?	○	○	-	-	✓

the cells of the plant seem to produce, or to produce in a more effective manner, the activating system required for 9/11 of the compounds classified as "problem carcinogens" for the majority of assays, including the Salmonella/S-9 system (Table 9).

In addition to the 37 compounds involved in the international project, we have tested the mutagenic effect of two mycotoxins, aflatoxin B1 and the trichotecin T2. Both substantially elevated the mutation frequency compared to a concurrent negative control (Table 11).

Table 11.

EFFECTS OF AFLATOXIN B1 and T2 MYCOTOXIN ON THE FREQUENCY OF EMBRYO MUTATIONS IN ARABIDOPSIS

Aflatoxin µg/ml	Pale Embryos % (Control Subtracted)
10	7.92
50	11.11
100	5.81
200	3.40
2000	3.04
T2 ml in 10 ml DMSO	
0.01	3.9
0.05	8.0
0.10	4.3
0.20	9.8
2.00	6.6

CONCLUSIONS

In comparison with the results of the 63 sets of test conducted by the international program including 42 chemicals, the Arabidopsis assays carried out with 37 compounds excelled by overall accuracy and sensitivity. The specificity of the Arabidopsis assays was, however, not as good as that of some other systems. This relatively low speficity can be attributed to the fact that several of the noncarcinogens were actually low efficiency carcinogens and the Arabidopsis mutation assay identified the true nature of these compounds.

All of the compounds were analyzed in several concentrations yet several gave inconclusive results. Therefore, furhter tests may be necessary before a definitive classification can be reached.

Obviously, the Arabidopsis assay cannot compete with many of the microbial assays in speed. Because of the high sensitivity of the assay it can serve, however, as an effective component of a battery of systems where high accuracy is necessary.

The exceptionally good sensitivity of the Arabidopsis assay can be attributed to the thousands of the target loci responding to the chemicals, and thus well representing the entire genome. Although chemicals act on the four nucleotides of the DNA or on the sugar-phosphate backbone of the molecule, the mutagenic effectiveness of an agent is influenced by a number of additional factors. Among these perhaps the conformation of the DNA (gene) may be more important than generally recognized (MacDermott 1982). Obviously, the conformational variety can be well represented only by multilocus forward mutation systems such as used in this study.

RECOMMENDATIONS

The Arabidopsis assay can be adopted as a short-term test for identifying carcinogens. It appears particularly useful for analyzing compounds which cannot be properly activated by S-9 system.

Further research is suggested for the study of the metabolic mechinery of Arabidopsis involved in the activation. Also, it would be highly desirable to verify by molecular methods the number of genes expressed during embryonal development of Arabidopsis. This can be done by determining the complexity of DNA transcribed before the seed coat matures.

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